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<b>(21) International Application Number:</b> PCT/US99/22924 <b>(22) International Filing Date:</b> 30 September 1999 (30.09.99)  <b>(30) Priority Data:</b> 09/164,193 30 September 1998 (30.09.98) US 09/163,833 30 September 1998 (30.09.98) US  <b>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications</b> US 09/164,193 (CIP) Filed on 30 September 1998 (30.09.98) US 09/163,833 (CIP) Filed on 30 September 1998 (30.09.98)  <b>(71) Applicant (for all designated States except US):</b> MILLENNIUM PHARMACEUTICALS, INC. [US/US]; 75 Sidney Street, Cambridge, MA 02139 (US).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> ACTON, Susan [US/US]; Apartment No. 7, 90 Bynner Street, Jamaica Plain, MA 02130 (US).		<b>(74) Agents:</b> MANDRAGOURAS, Amy, E.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US) et al.  <b>(81) Designated States:</b> AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> NOVEL PROTEIN PHOSPHATASE MOLECULES AND USES THEREFOR  <b>(57) Abstract</b> <p>Novel CSAPTP polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated, full-length CSAPTP proteins, the invention further provides isolated CSAPTP fusion proteins, antigenic peptides and anti-CSAPTP antibodies. The invention also provides CSAPTP nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which a CSAPTP gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.</p>		

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## NOVEL PROTEIN PHOSPHATASE MOLECULES AND USES THEREFOR

### Related Applications

This application claims priority to U.S. Patent Application Nos.: 09/164,193  
5 09/163,833 both filed on September 30, 1998, incorporated herein in their entirety by  
this reference.

### Background of the Invention

Phosphate tightly associated with protein has been known since the late  
10 nineteenth century. Since then, a variety of covalent linkages of phosphate to proteins  
have been found. The most common involve esterification of phosphate to serine and  
threonine, with smaller amounts being covalently linked to lysine, arginine, histidine,  
aspartic acid, glutamic acid, and cysteine. The occurrence of phosphorylated proteins  
implies the existence of one or more protein kinases capable of phosphorylating amino  
15 acid residues on proteins, and also of protein phosphatases capable of hydrolyzing  
phosphorylated amino acid residues on proteins.

Protein kinases play critical roles in the regulation of biochemical and  
morphological changes associated with cellular growth and division (D'Urso, G. et al.  
(1990) *Science* 250: 786-791; Birchmeier, C. et al. (1993) *Bioessays* 15: 185-189).  
20 They serve as growth factor receptors and signal transducers and have been implicated  
in cellular transformation and malignancy (Hunter, T. et al. (1992) *Cell* 70: 375-387;  
Posada, J. et al. (1992) *Mol. Biol. Cell* 3: 583-592; Hunter, T. et al. (1994) *Cell* 79: 573-  
582). For example, protein kinases have been shown to participate in the transmission  
of signals from growth-factor receptors (Sturgill, T. W. et al. (1988) *Nature* 344: 715-  
25 718; Gomez, N. et al. (1991) *Nature* 353: 170-173), control of entry of cells into mitosis  
(Nurse, P. (1990) *Nature* 344: 503-508; Maller, J. L. (1991) *Curr. Opin. Cell Biol.* 3:  
269-275) and regulation of actin bundling (Husain-Chishti, A. et al. (1988) *Nature* 334:  
718-721).

The overall level, in cells, of protein tyrosine phosphorylation, as well as the  
30 phosphorylated state of any given protein, arises from the balance of Protein Tyrosine  
Kinase (PTK) and Protein Tyrosine Phosphatase (PTPase) activities. Thus PTPases

have been proposed as key regulatory elements of cell growth control (Hunter, 1989, Cell 58:1013-1016).

PTKs were discovered and characterized more than one decade earlier than PTPases and in the last few years a large number of studies has led to the identification of many new PTPases and some of them have been accurately characterized. In addition, findings on the biological role of some PTPases in cells have recently been reported (Pondaven, 1991, *Adv Prot Phosphatases* 6:35-57). Current work suggests that PTKs and PTPases are equally important in many biological processes ranging from cell growth control to cell differentiation and development. In particular, the oncogenic potential of PTKs and the ability of PTPases to counteract PTK oncogenic activation by antiproliferative action suggests that the genes coding for PTPases, in many instances, may be considered tumor-suppressing genes or even anti-oncogenes.

The existence of PTPases was first predicted to explain the rapid loss of phosphorylation of *in vitro* phosphorylated membrane proteins (Carpenter *et al.*, 1979, *J Biol Chem* 254:4884-4891). The main PTPase in human placenta (PTP1B) was purified to homogeneity and sequenced (Tonks *et al.*, 1988, *J Biol Chem* 263:6722-2730; Charbonneau *et al.*, 1989, *PNAS USA* 86:5252-5256). Sequence homology between the catalytic domain of PTP1B and the leukocyte common antigen (LCA, or CD45) was demonstrated, indicating that PTPases can be considered a family of structurally related molecules.

The effects of many growth factors such as NGF, BDNF, NT3, FGF, insulin and IGF1 are known to be mediated by high-affinity receptors with tyrosine kinases activity (Fantl *et al. Annu. Rev. Biochem.*, 62 (1993) 453-481; Schlessinger and Ulrich *Neuron*, 9 (1992) 383-391; Ullrich and Schlessinger *Cell*, 61 (1990) 203-212). Expression of several tyrosine phosphatase genes has been detected in the brain (Jones *et al. J. Biol. Chem.*, 264 (1989) 7747-7753), including *RPTP $\alpha$*  (Kaplan *et al. Proc. Natl. Acad. Sci. USA*, 87 91990) 7000-7004; Sap *et al. Proc. Natl. Acad. Sci. USA*, 87 (1990) 6112-6116), *RNPTPX* (Guan *et al. Proc Natl. Acad. Sci. USA*, 87 (19910) 1501-1505), *STEP* (Lombroso *et al. Proc. Natl. Acad. Sci. USA*, 88 (1991) 7242-7246), *SH-PTP2* (Freeman *et al. Proc. Natl. Acad. Sci. USA*, 89 (1992) 11239-11243), *MPTP $\delta$*  (Mizuno

*et al. Mol. Cell. Biol.*, 13 (1993) 5513-5523), *DPTP99A* and *DPTP10D* (Yang *et al. Cell*, 67 (1991) 661-673).

Intraventricular administration of either NGF, BDNF, insulin or IGF1 prevents delayed neuronal death in the CA1 subfield of the hippocampus (Beck *et al. J. Cereb Blood Flow Metab.*, 14 (1994) 689-692; Shigeno *et al. J. Neurosci.*, 11 (1991) 2914-2919; Zhu and Auer *J. Cereb. Blood Flow Metab.*, 14 (1994) 237-242).

Tyrosine kinase inhibitors block the tyrosine phosphorylation of MAP kinase (Blenis *Proc. Natl. Acad. Sci. USA*, 90 (1993) 5889-5892; Pelech and Sanghera *Science*, 257 (1992) 1335-1356) and prevent delayed neuronal death after forebrain ischemia (Kindy *J. Cereb. Blood Flow Metab.*, 13 (1993) 372-377). During reperfusion after ischemia, tyrosine phosphorylation of proteins increases in the hippocampus but some proteins in the hippocampus are dephosphorylated (Campos-Gonzalez *J. Neurochem.*, 59 (1992) 1955-1958; Hu and Wieloch *J. Neurochem.*, 62 (1994) 1357-1367; Takano *et al. J. Cereb. Blood Flow Metab.*, 15 (1995) 33-41). These observations suggest that tyrosine phosphorylation plays an important role in the delayed neuronal death which occurs as a result of ischemia-reperfusion injury.

A number of PTPases, in addition to the hydrolytic activity on phosphotyrosine, show some phosphoserine/phosphothreonine phosphatase activity. These enzymes, mostly localized in the nucleus and referred to as dual-specificity PTPases (dsPTPases), are emerging as a subclass of PTPases acting as important regulators of cell cycle control and mitogenic signal transduction possibly by controlling the activity of signal transduction proteins like ERK. In fact, they appear responsible for *in vivo* nuclear dephosphorylation and inactivation of nuclear dephosphorylation and inactivation of MAP kinases (Alessi *et al.*, 1995, *Curr Biol* 5:195-283). These enzymes exhibit sequence identity to the vaccinia H-1 gene product, the first identified dsPTPase (Guan *et al.*, 1991, *Nature* 350:359-362). Several dsPTPases differing from each other in length have been identified. These enzymes and the other PTPase subclasses share an active site sequence motif showing only a limited sequence homology beyond this region.

Given the importance of such protein tyrosine phosphatases in the regulation of the cell cycle, there exists a need to identify novel protein tyrosine phosphatases which

function as modulators in the cell cycle such as the suppression of proliferation and whose aberrant function can result in disorders arising from improper cell cycle regulation such as cancer.

## 5 Summary of the Invention

The present invention is based, at least in part, on the discovery of novel nucleic acid molecules and proteins encoded by such nucleic acid molecules, referred herein as "Cardiovascular System Associated Protein Tyrosine Phosphatase" ("CSAPTP") proteins. The CSAPTP nucleic acid and protein molecules of the present invention are  
10 useful as modulating agents in regulating a variety of cellular processes, e.g., cardiac cellular processes. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding CSAPTP proteins or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of CSAPTP-encoding nucleic acids.

15 In one embodiment, a CSAPTP nucleic acid molecule of the invention is at least 35%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more homologous to a nucleotide sequence (e.g., to the entire length of the nucleotide sequence) including SEQ ID NO:1, SEQ ID NO:3, or a complement thereof. In another embodiment, a CSAPTP nucleic acid molecule is at least 40%, 45%, 50%,  
20 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more homologous to a nucleotide sequence including SEQ ID NO:4, SEQ ID NO:6, or a complement thereof. In yet another embodiment, a CSAPTP nucleic acid molecule is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more homologous to a nucleotide sequence including SEQ ID NO:7, SEQ ID NO:9, or a complement thereof.  
25 In a further embodiment, a CSAPTP nucleic acid molecule is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more homologous to a nucleotide sequence including SEQ ID NO:10, SEQ ID NO:12, or a complement thereof.

In a preferred embodiment, the isolated nucleic acid molecule includes the  
30 nucleotide sequence shown SEQ ID NO:1 or 3, or a complement thereof. In another embodiment, the nucleic acid molecule includes SEQ ID NO:3 and nucleotides 1-248 of

SEQ ID NO:1. In another embodiment, the nucleic acid molecule includes SEQ ID NO:3 and nucleotides 768-1315 of SEQ ID NO:1. In another preferred embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:1 or 3. In another preferred embodiment, the nucleic acid molecule comprises a fragment of at least 994 nucleotides of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or a complement thereof.

In another preferred embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:4 or 6, or a complement thereof. In another embodiment, the nucleic acid molecule includes SEQ ID NO:6 and nucleotides 790-1016 of SEQ ID NO:4. In another preferred embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:4 or 6. In another preferred embodiment, the nucleic acid molecule comprises a fragment of at least 626 nucleotides of the nucleotide sequence of SEQ ID NO:4, SEQ ID NO:6, or a complement thereof.

In another preferred embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:7 or 9, or a complement thereof. In another embodiment, the nucleic acid molecule includes SEQ ID NO:9 and nucleotides 628-814 of SEQ ID NO:7. In another embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:7 or 9. In another preferred embodiment, the nucleic acid molecule comprises a fragment of at least 531 nucleotides of the nucleotide sequence of SEQ ID NO:7, SEQ ID NO:9, or a complement thereof.

In another preferred embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:10 or 12, or a complement thereof. In another embodiment, the nucleic acid molecule includes SEQ ID NO:12 and nucleotides 664-928 of SEQ ID NO:10. In another preferred embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:10 or 12. In another preferred embodiment, the nucleic acid molecule comprises a fragment of at least 241 nucleotides of the nucleotide sequence of SEQ ID NO:10, SEQ ID NO:12, or a complement thereof.

In another embodiment, a CSAPTP nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO. 11. In a preferred embodiment, a CSAPTP nucleic acid molecule includes a

nucleotide sequence encoding a protein having an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 78%, 79%, 80%, 85%, 90%, 95%, 99% or more homologous to an amino acid sequence including SEQ ID NO:2 (e.g., the entire amino acid sequence of SEQ ID NO:2). In another preferred embodiment, a CSAPTP nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 10%, 15%, 20%, 22%, 23%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more homologous to an amino acid sequence including SEQ ID NO:5 (e.g., the entire amino acid sequence of SEQ ID NO:5). In yet another preferred embodiment, a CSAPTP nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 10%, 15%, 20%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more homologous to an amino acid sequence including SEQ ID NO:8 (e.g., the entire amino acid sequence of SEQ ID NO:8). In another preferred embodiment, a CSAPTP nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 30%, 35%, 40%, 42%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to an amino acid sequence including SEQ ID NO:11 (e.g., the entire amino acid sequence of SEQ ID NO:11).

In another preferred embodiment, an isolated nucleic acid molecule encodes the amino acid sequence of a human CSAPTP. In yet another preferred embodiment, the nucleic acid molecule includes a nucleotide sequence encoding a protein which includes the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11. In yet another preferred embodiment, the nucleic acid molecule includes a nucleotide sequence encoding a protein having the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO: 8, or SEQ ID NO:11.

Another embodiment of the invention features nucleic acid molecules, preferably CSAPTP nucleic acid molecules, which specifically detect CSAPTP nucleic acid molecules relative to nucleic acid molecules encoding non-CSAPTP proteins. For example, in one embodiment, such a nucleic acid molecule is at least 240, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100 nucleotides in length and hybridizes under stringent conditions to a nucleic acid

molecule comprising the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, or a complement thereof.

In a particularly preferred embodiment, the nucleic acid molecule comprises a fragment of at least 994 nucleotides of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or a complement thereof. In preferred embodiments, the nucleic acid molecules are at least 15 (e.g., contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-17 and 1011-1315 of SEQ ID NO:1. In other preferred embodiments, the nucleic acid molecules comprise nucleotides 1-17 and 1011-1315 of SEQ ID NO:1. In other preferred embodiments, the nucleic acid molecules consist of nucleotides 1-17 and 1011-1315 of SEQ ID NO:1.

In another particularly preferred embodiment, the nucleic acid molecule comprises a fragment of at least 626 nucleotides of the nucleotide sequence of SEQ ID NO:4, SEQ ID NO:6, or a complement thereof. In preferred embodiments, the nucleic acid molecules are at least 15 (e.g., contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-342 of SEQ ID NO:4. In preferred embodiments, the nucleic acid molecules are at least 15 (e.g., contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1002-1016 of SEQ ID NO:4. In preferred embodiments, the nucleic acid molecules comprise nucleotides 1002-1016 of SEQ ID NO:4. In preferred embodiments, the nucleic acid molecules consist of nucleotides 1002-1016 of SEQ ID NO:4.

In another particularly preferred embodiment, the nucleic acid molecule comprises a fragment of at least 531 nucleotides of the nucleotide sequence of SEQ ID NO:7, SEQ ID NO:9, or a complement thereof. In preferred embodiments, the nucleic acid molecules are at least 15 (e.g., contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-103 and 774-814 of SEQ ID NO:7. In preferred embodiments, the nucleic acid molecules comprise nucleotides 1-103 and 774-814 of SEQ ID NO:7. In preferred embodiments, the nucleic acid molecules consist of nucleotides 1-103 and 774-814 of SEQ ID NO:7.

In another particularly preferred embodiment, the nucleic acid molecule comprises a fragment of at least 241 nucleotides of the nucleotide sequence of SEQ ID NO:10, SEQ ID NO:12, or a complement thereof. In preferred embodiments, the

nucleic acid molecules are at least 15 (e.g., contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-471 and 881-928 of SEQ ID NO:10. In other preferred embodiments, the nucleic acid molecules comprise nucleotides 1-471 and 881-928 of SEQ ID NO:1. In other preferred embodiments, the  
5 nucleic acid molecules consist of nucleotides 1-471 and 881-928 of SEQ ID NO:1.

In other preferred embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide which includes the amino acid sequence of SEQ ID NO:2, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule which includes SEQ ID NO:1 or SEQ ID NO:3 under stringent conditions. In other  
10 preferred embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide which includes the amino acid sequence of SEQ ID NO:5, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule which includes SEQ ID NO:4 or SEQ ID NO:6 under stringent conditions. In other preferred  
embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a  
15 polypeptide which includes the amino acid sequence of SEQ ID NO:8, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule which includes SEQ ID NO:7 or SEQ ID NO:9 under stringent conditions. In other preferred embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide  
which includes the amino acid sequence of SEQ ID NO:11, wherein the nucleic acid  
20 molecule hybridizes to a nucleic acid molecule which includes SEQ ID NO:10 or SEQ ID NO:12 under stringent conditions.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to a CSAPTP nucleic acid molecule, e.g., the coding strand of a CSAPTP nucleic acid molecule.

25 Another aspect of the invention provides a vector comprising a CSAPTP nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. The invention also provides a method for producing a protein, preferably a CSAPTP protein, by culturing in a suitable medium, a host cell, e.g., a mammalian host  
30 cell such as a non-human mammalian cell, of the invention containing a recombinant expression vector, such that the protein is produced.



Another aspect of this invention features isolated or recombinant CSAPTP proteins and polypeptides. In one embodiment, the isolated protein, preferably a CSAPTP-1 protein, includes at least one CSAPTP-1 unique N-terminal domain and at least one phosphatase active domain. In another embodiment, the isolated protein, preferably a CSAPTP-1 protein, includes at least one CSAPTP-1 unique N-terminal domain and at least one phosphatase active domain and has an amino acid sequence which is at least 50%, 55%, 60%, 65%, 70%, 75%, 78%, 79%, 80%, 85%, 90%, 95%, 99% or more homologous to an amino acid sequence including SEQ ID NO:2. In yet another embodiment, the isolated protein, preferably a CSAPTP-1 protein, includes at least one CSAPTP-1 unique N-terminal domain and at least one phosphatase active domain and is expressed and/or functions in cells of the cardiovascular system. In an even further embodiment, the isolated protein, preferably a CSAPTP-1 protein, includes at least one CSAPTP-1 unique N-terminal domain and at least one phosphatase active domain and plays a role in signaling pathways associated with cellular growth, e.g., signaling pathways associated with cell cycle regulation. In another embodiment, the isolated protein, preferably a CSAPTP-1 protein, includes at least one CSAPTP-1 unique N-terminal domain and at least one phosphatase active domain and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3.

In another embodiment, the isolated protein, preferably a CSAPTP-2 protein, includes at least one CSAPTP-2 unique N-terminal domain and at least one phosphatase active domain. In another embodiment, the isolated protein, preferably a CSAPTP-2 protein, includes at least one CSAPTP-2 unique N-terminal domain and at least one phosphatase active domain and has an amino acid sequence which is at least 10%, 15%, 20%, 22%, 23%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more homologous to an amino acid sequence including SEQ ID NO:5. In yet another embodiment, the isolated protein, preferably a CSAPTP-2 protein, includes at least one CSAPTP-2 unique N-terminal domain and at least one phosphatase active domain and is expressed and/or functions in cells of the cardiovascular system. In an even further embodiment, the isolated protein, preferably a

CSAPTP-2 protein, includes at least one CSAPTP-2 unique N-terminal domain and at least one phosphatase active domain and plays a role in signaling pathways associated with cellular growth, e.g., signaling pathways associated with cell cycle regulation. In another embodiment, the isolated protein, preferably a CSAPTP-2 protein, includes at least one CSAPTP-2 unique N-terminal domain and at least one phosphatase active domain and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:4 or SEQ ID NO:6.

In yet another embodiment, the isolated protein, preferably a CSAPTP-3 protein, includes at least one CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain. In another embodiment, the isolated protein, preferably a CSAPTP-3 protein, includes at least one CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain and has an amino acid sequence which is at least 10%, 15%, 20%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more homologous to an amino acid sequence including SEQ ID NO:8. In yet another embodiment, the isolated protein, preferably a CSAPTP-3 protein, includes at least one CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain and is expressed and/or functions in cells of the cardiovascular system. In an even further embodiment, the isolated protein, preferably a CSAPTP-3 protein, includes at least one CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain and plays a role in signaling pathways associated with cellular growth, e.g., signaling pathways associated with cell cycle regulation. In another embodiment, the isolated protein, preferably a CSAPTP-3 protein, includes at least one CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:7 or SEQ ID NO:9.

In yet another embodiment, the isolated protein, preferably a CSAPTP-4 protein, includes at least one CSAPTP-4 unique N-terminal domain and at least one phosphatase active domain. In another embodiment, the isolated protein, preferably a CSAPTP-4 protein, includes at least one CSAPTP-4 unique N-terminal domain and at least one

phosphatase active domain and has an amino acid sequence which is at least 30%, 35%, 40%, 42%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or more homologous to an amino acid sequence including SEQ ID NO:11. In yet another embodiment, the isolated protein, preferably a CSAPTP-4 protein, includes at least one CSAPTP-4 unique N-terminal domain and at least one phosphatase active domain and is expressed and/or functions in cells of the cardiovascular system. In an even further embodiment, the isolated protein, preferably a CSAPTP-4 protein, includes at least one CSAPTP-4 unique N-terminal domain and at least one phosphatase active domain and plays a role in signaling pathways associated with cellular growth, e.g., signaling pathways associated with cell cycle regulation. In another embodiment, the isolated protein, preferably a CSAPTP-4 protein, includes at least one CSAPTP-4 unique N-terminal domain and at least one phosphatase active domain and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:10 or SEQ ID NO:12.

In another embodiment, the isolated protein, preferably a CSAPTP protein, has an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11. In a preferred embodiment, the protein, preferably a CSAPTP protein, has an amino acid sequence at least 79%, 23%, 24%, 42% or more homologous to an amino acid sequence including SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, respectively (e.g., the entire amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11). In another embodiment, the invention features fragments of the proteins having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, wherein the fragment comprises at least 15 amino acids (e.g., contiguous amino acids) of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, respectively. In another embodiment, the protein, preferably a CSAPTP protein, has the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11.

Another embodiment of the invention features an isolated protein, preferably a CSAPTP protein, which is encoded by a nucleic acid molecule having a nucleotide

sequence at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more homologous to a nucleotide sequence (e.g., to the entire length of the nucleotide sequence) including SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:12, respectively, or  
5 a complement thereof. This invention further features an isolated protein, preferably a CSAPTP protein, which is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:12,  
10 or a complement thereof.

The proteins of the present invention or biologically active portions thereof, can be operatively linked to a non-CSAPTP polypeptide (e.g., heterologous amino acid sequences) to form fusion proteins. The invention further features antibodies, such as monoclonal or polyclonal antibodies, that specifically bind proteins of the invention,  
15 preferably CSAPTP proteins. In addition, the CSAPTP proteins or biologically active portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting the presence of a CSAPTP nucleic acid molecule, protein or polypeptide in a biological  
20 sample by contacting the biological sample with an agent capable of detecting a CSAPTP nucleic acid molecule, protein or polypeptide such that the presence of a CSAPTP nucleic acid molecule, protein or polypeptide is detected in the biological sample.

In another aspect, the present invention provides a method for detecting the presence of CSAPTP activity in a biological sample by contacting the biological sample  
25 with an agent capable of detecting an indicator of CSAPTP activity such that the presence of CSAPTP activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating CSAPTP activity comprising contacting a cell capable of expressing CSAPTP with an agent that  
30 modulates CSAPTP activity such that CSAPTP activity in the cell is modulated. In one embodiment, the agent inhibits CSAPTP activity. In another embodiment, the agent

stimulates CSAPTP activity. In one embodiment, the agent is an antibody that specifically binds to a CSAPTP protein. In another embodiment, the agent modulates expression of CSAPTP by modulating transcription of a CSAPTP gene or translation of a CSAPTP mRNA. In yet another embodiment, the agent is a nucleic acid molecule  
5 having a nucleotide sequence that is antisense to the coding strand of a CSAPTP mRNA or a CSAPTP gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant CSAPTP protein or nucleic acid expression or activity by administering an agent which is a CSAPTP modulator to the  
10 subject. In one embodiment, the CSAPTP modulator is a CSAPTP protein. In another embodiment the CSAPTP modulator is a CSAPTP nucleic acid molecule. In yet another embodiment, the CSAPTP modulator is a peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the disorder characterized by aberrant CSAPTP protein or nucleic acid expression is an immune disorder, an anti-proliferative disorder, a  
15 proliferative disorder, e.g., cancer, for example sporadic cancers e.g., brain, breast and prostate; inherited autosomal-dominant cancer, e.g., Cowden's syndrome; renal and lung carcinomas; a metabolic disorder, e.g., diabetes, for example, impaired dephosphorylation of both the insulin receptor and insulin receptor substrate-1; viral pathogenesis, e.g., cancer, for example, adenovirus E1A-mediated cell proliferation; e.g.,  
20 Boubonic Plague, for example, pathogenic *Yersinia pestis* viral PTPase dephosphorylation of host phospho-proteins; a neural disorder; a cardiovascular disorder, e.g., congestive heart failure, or a disorder arising from improper dephosphorylation of phosphorylated protein.

The present invention also provides a diagnostic assay for identifying the  
25 presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a CSAPTP protein; (ii) mis-regulation of the gene; and (iii) aberrant post-translational modification of a CSAPTP protein, wherein a wild-type form of the gene encodes a protein with a CSAPTP activity.

In another aspect the invention provides a method for identifying a compound  
30 that binds to or modulates the activity of a CSAPTP protein, by providing an indicator composition comprising a CSAPTP protein having CSAPTP activity, contacting the

indicator composition with a test compound, and determining the effect of the test compound on CSAPTP activity in the indicator composition to identify a compound that modulates the activity of a CSAPTP protein.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

#### Brief Description of the Drawings

*Figure 1* depicts the cDNA sequence and predicted amino acid sequence of human CSAPTP-1. The nucleotide sequence corresponds to nucleic acids 1 to 1315 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 173 of SEQ ID NO:2. The coding region without the 5' and 3' untranslated regions of the human CSAPTP-1 gene is shown in SEQ ID NO:3.

*Figure 2* depicts the cDNA sequence and predicted amino acid sequence of human CSAPTP-2. The nucleotide sequence corresponds to nucleic acids 1 to 1016 of SEQ ID NO:4. The amino acid sequence corresponds to amino acids 1 to 263 of SEQ ID NO:5. The coding region without the 3' untranslated region of the human CSAPTP-2 gene is shown in SEQ ID NO:6.

*Figure 3* depicts the cDNA sequence and predicted amino acid sequence of human CSAPTP-3. The nucleotide sequence corresponds to nucleic acids 1 to 814 of SEQ ID NO:7. The amino acid sequence corresponds to amino acids 1 to 209 of SEQ ID NO:8. The coding region without the 3' untranslated region of the human CSAPTP-3 gene is shown in SEQ ID NO:9.

*Figure 4* depicts a global alignment between the CSAPTP-1 DNA sequence and the human protein tyrosine phosphatase DNA sequence (Accession No. AAB40597). This alignment were generated utilizing the ALIGN program with the following parameter setting: PAM120, gap penalties: -12/-4 (Myers, E. and Miller, W. (1988) "Optimal Alignments in Linear Space" CABIOS 4:11-17). The results showed a 38.7% identity between the two sequences.

Figure 5 depicts a global alignment between the CSAPTP-1 protein sequence and the human protein tyrosine phosphatase protein sequence (Accession No. AAB40597). This alignment were generated utilizing the ALIGN program with the following parameter setting: PAM120, gap penalties: -12/-4 (Myers, E. and Miller, W. (1988) "Optimal Alignments in Linear Space" CABIOS 4:11-17). The results showed a 78.6% identity between the two sequences.

Figure 6 depicts a global alignment between the CSAPTP-2 protein sequence and the human dual specificity phosphatase (SwissProt: P51452) protein sequence. This alignment were generated utilizing the ALIGN program with the following parameter setting: PAM120, gap penalties: -12/-4 (Myers, E. and Miller, W. (1988) "Optimal Alignments in Linear Space" CABIOS 4:11-17). The results showed a 22.5% identity between the two sequences.

Figure 7 depicts a global alignment between the CSAPTP-3 protein sequence and the SwissProt: Z68315 protein sequence. This alignment were generated utilizing the ALIGN program with the following parameter setting: PAM120, gap penalties: -12/-4 (Myers, E. and Miller, W. (1988) "Optimal Alignments in Linear Space" CABIOS 4:11-17). The results showed a 24% identity between the two sequences.

Figure 8 is a list of genes whose expression is altered by the expression of CSAPTP-1 in C2C12 cells.

Figure 9 depicts the cDNA sequence and predicted amino acid sequence of human CSAPTP-4. The nucleotide sequence corresponds to nucleic acids 1 to 912 of SEQ ID NO:10. The amino acid sequence corresponds to amino acids 1 to 221 of SEQ ID NO:11. The coding region without the 5' and 3' untranslated regions of the human CSAPTP-4 gene is shown in SEQ ID NO:12.

Figure 10 depicts a global alignment between the human CSAPTP-4 amino acid sequence and dual specificity protein phosphatase (SwissProt No: P51452) amino acid sequence. This alignment were generated utilizing the ALIGN program with the following parameter setting: PAM120, gap penalties: -12/-4 (Myers, E. and Miller, W. (1988) "Optimal Alignments in Linear Space" CABIOS 4:11-17). The results showed a 42% identity between the two sequences.

### Detailed Description of the Invention

The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as "Cardiovascular System Associated Protein Tyrosine Phosphatase" or "CSAPTP" nucleic acid and polypeptide molecules, which play a role in or function in a variety of cellular processes, e.g., proliferation, differentiation, anti-proliferative mechanisms, immune responses, viral replication in a host, viral pathogenicity, neuroprotective responses, insulin responses, and cardiac cellular processes. In one embodiment, the CSAPTP molecules of the present invention modulate the activity of one or more proteins involved in a cellular proliferative and/or differentiative disorder, e.g., cancer. In another embodiment, the CSAPTP molecules of the present invention modulate the activity of one or more proteins involved in an immune cell disorder. In another embodiment, the CSAPTP molecules of the present invention modulate the activity of one or more proteins involved in viral replication and/or viral pathogenesis in a host. In another embodiment, the CSAPTP molecules of the present invention modulate the activity of one or more proteins involved in a neuroprotective response, e.g., neuronal response to ischemic injury. In another embodiment, the CSAPTP molecules of the present invention modulate the activity of one or more proteins involved in a metabolic disorder, e.g., diabetes. In one embodiment, the CSAPTP molecules of the present invention modulate the activity of one or more proteins involved in a cardiovascular disorder, e.g., congestive heart failure. In another embodiment, the CSAPTP molecules of the present invention are capable of modulating the phosphorylation state of a CSAPTP molecule or one or more proteins involved in cellular growth or differentiation, e.g., cardiac cell growth or differentiation.



As used herein, the term "protein tyrosine phosphatase" or "PTPase" includes a protein or polypeptide (e.g., an enzyme) which is capable of facilitating (e.g., catalyzing) the removal of a phosphate group attached to a tyrosine, serine or threonine residue of a protein or polypeptides (e.g., a phosphoprotein). As referred to herein, a "protein tyrosine phosphatase" includes at least one catalytic domain having a specificity for dephosphorylating tyrosine residues or both serine/threonine residues and tyrosine residues (e.g., the dual specificity PTPases) and including at least about 200-400 amino acid residues in length, preferably about 200-300 amino acid residues in length, and more preferably about 250-300 amino acid residues in length. Phosphatases of the present invention preferably include a catalytic domain which includes at least one conserved motif or subdomain having at least about 30-50 amino acid residues, more preferably 40 amino acid residues which are conserved among PTPases. PTPases can be either soluble or membrane bound (see e.g., Brautigan *Biochem. biophys. Acta*, 1114 (1992) 63-77; Charbonneau *Ann. Rev. Cell Biol.*, 8 (1992) 463-493; Fisher *et al. Science*, 253 (1991) 401-406; Hunter *Cell*, 58 (1989) 1013-1016).

Membrane bound PTPases typically contain receptor-like extracellular regions connected to the intracellular (catalytic) domains by a short transmembrane segment (Streuli and Saito, 1993, *Adv Prot Phosphatases* 7:67-94). The non-transmembrane (cytoplasmic) PTPases typically include at least one catalytic domain (Koch *et al.*, 1991, *Science* 252:668-674).

As used herein, the term "protein kinase" includes a protein or polypeptide which is capable of modulating its own phosphorylation state or the phosphorylation state of another protein or polypeptide. Protein kinases can have a specificity for (i.e., a specificity to phosphorylate) serine/threonine residues, tyrosine residues, or both serine/threonine and tyrosine residues, e.g., the dual specificity kinases.

As used herein, the term "cardiovascular disorder" includes a disease, disorder, or state involving the cardiovascular system, e.g., the heart, the blood vessels, and/or the blood. A cardiovascular disorder can be caused by an imbalance in arterial pressure, a malfunction of the heart, or an occlusion of a blood vessel, e.g., by a thrombus. Examples of such disorders include hypertension, atherosclerosis, coronary artery spasm, coronary artery disease, valvular disease, arrhythmias, cardiomyopathies (e.g.,

dilated cardiomyopathy, idiopathic cardiomyopathy), arteriosclerosis, ischemia  
reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling,  
ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia,  
bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart  
5 disease, atrial fibrillation, long-QT syndrome, congestive heart failure, sinus node  
disfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter,  
myocardial infarction, cardiac hypertrophy, and coronary artery spasm.

As used herein, the term "congestive heart failure" includes a condition  
characterized by a diminished capacity of the heart to supply the oxygen demands of the  
10 body. Symptoms and signs of congestive heart failure include diminished blood flow to  
the various tissues of the body, accumulation of excess blood in the various organs, e.g.,  
when the heart is unable to pump out the blood returned to it by the great veins,  
exertional dyspnea, fatigue, and/or peripheral edema, e.g., peripheral edema resulting  
from left ventricular dysfunction. Congestive heart failure may be acute or chronic. The  
15 manifestation of congestive heart failure usually occurs secondary to a variety of cardiac  
or systemic disorders that share a temporal or permanent loss of cardiac function.  
Examples of such disorders include hypertension, coronary artery disease, valvular  
disease, and cardiomyopathies, e.g., hypertrophic, dilative, or restrictive  
cardiomyopathies. Congestive heart failure is described in, for example, Cohn J.N. et al.  
20 (1998) *American Family Physician* 57:1901-04, the contents of which are incorporated  
herein by reference.

As used herein, the term "cardiac cellular processes" includes intra-cellular or  
inter-cellular processes involved in the functioning of the heart. Cellular processes  
involved in the nutrition and maintenance of the heart, the development of the heart, or  
25 the ability of the heart to pump blood to the rest of the body are intended to be covered  
by this term. Such processes include, for example, cardiac muscle contraction,  
distribution and transmission of electrical impulses, and cellular processes involved in  
the opening and closing of the cardiac valves. The term "cardiac cellular processes"  
further includes processes such as the transcription, translation and post- translational  
30 modification of proteins involved in the functioning of the heart, e.g., myofilament

specific proteins, such as troponin I, troponin T, myosin light chain 1 (MLC1), and  $\alpha$ -actinin.

As used herein, a "cellular proliferative disorder" includes a disorder, disease, or condition characterized by a deregulated, e.g., upregulated or downregulated, growth  
5 response. As used herein, a "cellular differentiative disorder" includes a disorder, disease, or condition characterized by aberrant or deficient cellular differentiation.

The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as CSAPTP protein and nucleic acid molecules, which  
10 comprise a family of molecules having certain conserved structural and functional features. The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide  
15 sequence homology as defined herein. Such family members can be naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other, distinct proteins of human origin or  
alternatively, can contain homologues of non-human origin. Members of a family may also have common functional characteristics.

One embodiment of the invention features CSAPTP nucleic acid molecules, preferably human CSAPTP molecules, e.g., CSAPTP-1, CSAPTP-2, CSAPTP-3 and  
20 CSAPTP-4, which were identified from cDNA libraries made from hearts of patients with congestive heart failure (CHF) of ischemic and idiopathic origin. The CSAPTP nucleic acid and protein molecules of the invention are described in further detail in the following subsections.

#### 25 A. The CSAPTP-1 Nucleic Acid and Protein Molecules

One embodiment of the invention features CSAPTP-1 proteins and nucleic acid molecules which have sequence similarity with protein-tyrosine phosphatases. Accordingly, CSAPTP-1 polypeptides of the invention may interact with (e.g., bind to)  
30 at least one ligand which is a phosphorylated tyrosine of a protein and, thus, may be involved in the regulation of proliferation, anti-proliferative mechanisms, immune

responses, viral replication in a host, viral pathogenicity, neuroprotective responses, insulin responses, and cardiac cellular processes.

In one embodiment, the isolated proteins of the present invention, preferably CSAPTP-1 proteins, are identified based on the presence of a CSAPTP-1 unique N-terminal domain and at least one phosphatase active domain. As used herein, a "CSAPTP-1 unique N-terminal domain" includes a protein domain which is at least about 60-110 amino acid residues in length, preferably at least 60-100 amino acid residues in length, more preferably at least 60-90, or at least 60-80, or preferably 67 amino acid residues in length. In another embodiment, a CSAPTP-1 unique N-terminal domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a CSAPTP-1 unique N-terminal domain of a human CSAPTP-1 sequence set forth in SEQ ID NO:2 (e.g., amino acid residues 1-67 of the amino acid sequence as set forth in SEQ ID NO:2). As further defined herein, a CSAPTP-1 unique N-terminal domain of a CSAPTP-1 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-1 protein family. In a preferred embodiment, a CSAPTP-1 unique N-terminal domain has amino acid residues 1-67 of SEQ ID NO:2.

In another embodiment of the invention, a CSAPTP-1 family member is identified based on the presence of a phosphatase active domain. As used herein, a "phosphatase active domain" includes a protein domain which is at least 60-110 amino acid residues in length, preferably at least 70-110 amino acid residues in length, and more preferably at least 75-110, or at least 80-110, or preferably 105 amino acid residues in length, which is conserved in phosphatases which dephosphorylate tyrosine, serine, or threonine residues and is found in the catalytic domain of PTPases. A phosphatase active domain is capable of facilitating (e.g., catalyzing) the removal of a phosphate group attached to a tyrosine, serine or threonine residue of a protein (e.g., a phosphoprotein).

In one embodiment, a phosphatase active domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a phosphatase active domain of a human CSAPTP-1 sequence set forth in SEQ ID NO:2. In another embodiment, a phosphatase active domain has amino acid

residues 68-173 of the amino acid sequence as set forth in SEQ ID NO:2. Phosphatase active domains are described in, for example, Charbonneau, H. and Tonks, N.K. (1992) *Ann. Rev. Cell Biol.* 8:463-493, and Zhang, Z (1998) *Crit. Rev. Biochem. Mol. Biol.* 33(1):1-52, the contents of which are incorporated herein by reference. As further  
5 defined herein, a phosphatase active domain of a CSAPTP-1 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-1 protein family.

In another embodiment of the invention, a CSAPTP-1 family member is identified based on the presence of at least one phosphatase extended catalytic active  
10 domain within a phosphatase active domain. As used herein, a "phosphatase extended catalytic active domain" includes a protein domain which is at least 5-50 amino acid residues in length, preferably at least 10-40 amino acid residues in length, more preferably at least 15-25, or at least 20-22, or preferably 21 amino acid residues in length which is conserved in phosphatases which dephosphorylate tyrosine, serine, or threonine  
15 residues and found in the catalytic domain of PTPases. A phosphatase extended catalytic active domain is capable of facilitating (e.g., catalyzing) the removal of a phosphate group attached to a tyrosine, serine or threonine residue of a protein (e.g., a phosphoprotein). Preferably, the phosphatase extended catalytic active domain includes the following amino acid consensus sequence (VXVHCXAGXSRSTX (3) AYLM, X=  
20 any amino acid). Phosphatase extended catalytic active domains are described in, for example, Keyse, S.M. (1995) *Biochimica et Biophysica Acta* 1265:152-160, the contents of which are incorporated herein by reference. In another embodiment, a phosphatase extended catalytic active domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a  
25 phosphatase extended catalytic active domain of a human CSAPTP-1 sequence set forth in SEQ ID NO:2 (e.g., amino acid residues 100-120). In another embodiment, a phosphatase extended catalytic active domain has amino acid residues 100-120 of the amino acid sequence as set forth in SEQ ID NO:2. Phosphatase extended catalytic active domains are described in, for example, Ramponi G. and Stefani, M (1997) *Int. J.*  
30 *Biochem. Cell Biol.* 29(2):279-292, the contents of which are incorporated herein by reference. As further defined herein, a phosphatase extended catalytic active domain of

a CSAPTP-1 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-1 protein family.

In another embodiment of the invention, a CSAPTP-1 family member is  
5 identified based on the presence of at least one phosphatase catalytic active domain within a phosphatase active domain. As used herein, a "phosphatase catalytic active domain" includes a protein domain which is at least 5-20 amino acid residues in length, preferably at least 7-15 amino acid residues in length, and more preferably at least 8-12, or at least 9-11, or preferably 10 amino acid residues in length which is conserved in  
10 phosphatases which dephosphorylate tyrosine, serine, or threonine residues and found in the catalytic domain of PTPases. A phosphatase catalytic active domain is capable of facilitating (e.g., catalyzing) the removal of a phosphate group attached to a tyrosine, serine or threonine residue of a protein (e.g., a phosphoprotein). Preferably, in one embodiment, the phosphatase catalytic active domain includes the following amino acid  
15 consensus sequence ([I/V] HCXAGXXR [S/T] X= any amino acid). In another embodiment, a phosphatase catalytic active domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a phosphatase catalytic active domain of a human CSAPTP-1 sequence set forth in SEQ ID NO:2 (e.g., amino acid residues 102-111). Preferably, a phosphatase  
20 catalytic active domain has amino acid residues 102-111 of the amino acid sequence as set forth in SEQ ID NO:2. Phosphatase catalytic active domains are described in, for example, Keyse, S.M. (1995) *Biochimica et Biophysica Acta* 1265:152-160, the contents of which are incorporated herein by reference. As further defined herein, a phosphatase catalytic active domain of a CSAPTP-1 protein family member, however, is not  
25 sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-1 protein family.

In another embodiment of the invention, a CSAPTP-1 family member is identified based on the presence of at least one phosphatase catalytic core active domain within a phosphatase active domain. As used herein, a "phosphatase catalytic core active  
30 domain" includes a protein domain which is at least 5-15 amino acid residues in length, preferably at least 7-11, or at least 8-10, or preferably 9 amino acid residues in length

which is conserved in phosphatases which dephosphorylate tyrosine, serine, or threonine residues and found in the catalytic domain of PTPases. A phosphatase catalytic core active domain is capable of facilitating (e.g., catalyzing) the removal of a phosphate group attached to a tyrosine, serine or threonine residue of a protein (e.g., a phosphoprotein). Preferably, in one embodiment, the phosphatase catalytic core active domain includes the following amino acid consensus sequence (H/V)C(X)<sub>5</sub>R(S/T), X= any amino acid). In another embodiment, a phosphatase catalytic core active domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a phosphatase catalytic core active domain of a human CSAPTP-1 sequence set forth in SEQ ID NO:2 (e.g., amino acid residues 103-111). Preferably, a phosphatase catalytic core active domain has amino acid residues 103-111 of the amino acid sequence as set forth in SEQ ID NO:2. Phosphatase catalytic core active domains are described in, for example, Misra-Press, A. et al (1995) *J Biol. Chem.* 270(24):14587-14596, the contents of which are incorporated herein by reference. As further defined herein, a phosphatase catalytic core active domain of a CSAPTP-1 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-1 protein family.

In another embodiment of the invention, a CSAPTP-1 family member is identified based on the presence of at least one phosphatase catalytic invariant core active domain within the phosphatase active domain. As used herein, a "phosphatase catalytic invariant core active domain" includes a protein domain which is at least 4-15 amino acid residues in length, preferably at least 5-11 amino acid residues in length, and more preferably at least 6-10, or at least 8-9, or preferably 7 amino acid residues in length which is conserved in phosphatases which dephosphorylate tyrosine, serine, or threonine residues and found in the catalytic domain of PTPases. A phosphatase catalytic invariant core active domain is capable of facilitating (e.g., catalyzing) the removal of a phosphate group attached to a tyrosine, serine or threonine residue of a protein (e.g., a phosphoprotein). Preferably, the phosphatase catalytic invariant core active domain includes the following amino acid consensus sequence (C(X))<sub>5</sub>R, X= any amino acid). In another embodiment, a phosphatase catalytic invariant core active

domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a phosphatase catalytic invariant core active domain of a human CSAPTP-1 sequence set forth in SEQ ID NO:2 (e.g., amino acid residues 104-110). In another embodiment, a phosphatase catalytic invariant core  
5 active domain has amino acid residues 104-110 of the amino acid sequence as set forth in SEQ ID NO:2. Phosphatase catalytic invariant core active domains are described in, for example, Misra-Press, A. et al (1995) *J Biol. Chem.* 270(24):14587-14596, the contents of which are incorporated herein by reference. As further defined herein, a phosphatase catalytic invariant core active domain of a CSAPTP-1 protein family  
10 member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-1 protein family.

In another embodiment of the invention, a CSAPTP-1 family member is identified based on the presence of an intradomain disulfide bond consensus sequence. As used herein, an "intradomain disulfide bond consensus sequence" includes a protein  
15 domain which is at least 3-20 amino acid residues in length, preferably at least 3-15 amino acid residues in length, and more preferably at least 5-9, or at least 6-8, or preferably 7 amino acid residues in length, and is the site of cysteine-cysteine bonding within a single protein molecule. In one embodiment, an intradomain disulfide bond consensus sequence has at least 65-95%, preferably at least 70-90%, and more  
20 preferably at least 75-85% homology with the amino acid sequence of an intradomain disulfide bond consensus sequence of a human CSAPTP-1 sequence set forth in SEQ ID NO:2 (e.g., amino acid residues 97-103). In another embodiment, an intradomain disulfide bond consensus sequence has amino acid residues 97-103 of the amino acid sequence as set forth in SEQ ID NO:2. Intradomain disulfide bond consensus sequence  
25 domains are described in, for example, Beck, S. and Barrel, B.G. (1988) *Nature* 331:269-272, the contents of which are incorporated herein by reference. As further defined herein, an intradomain disulfide bond consensus sequence of a CSAPTP-1 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-1 protein  
30 family.



In another embodiment, CSAPTP-1 family members include at least 1, 2, or more Protein kinase C (PKC) phosphorylation sites. PKC phosphorylation sites can be found at least at residues 13-15 and 165-167 of SEQ ID NO:2. CSAPTP-1 family members can further include at least 1, 2, 3, 4 or more Casein kinase II phosphorylation sites. Casein kinase II phosphorylation sites can be found at least at residues 32-35, 56-59, 64-67 and 147-150 of SEQ ID NO:2. CSAPTP-1 family members can further include at least one tyrosine kinase phosphorylation site. Tyrosine kinase phosphorylation sites can be found at least at residues 47-53 of SEQ ID NO:2. CSAPTP-1 family members can further include at least 1, 2 or more N-glycosylation sites. N-glycosylation sites can be found at least at residues 27-30 of SEQ ID NO:2. CSAPTP-1 family members can further include at least 1, 2 or more N-myristoylation sites. N-myristoylation sites can be found at least at residues 97-102 and 139-144 of SEQ ID NO:2. CSAPTP-1 family members can further include at least 1, 2 or more homeobox domains. Homeobox domains can be found at least at residues 134-152 of SEQ ID NO:2.

Isolated proteins of the present invention, preferably CSAPTP-1 proteins, have an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2 or are encoded by a nucleotide sequence which includes a nucleotide sequence sufficiently homologous to SEQ ID NO:1, SEQ ID NO:3. As used herein, the term "sufficiently homologous" includes a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30-40% homology, preferably 40-50% homology, more preferably 50-60%, and even more preferably 60-70%, 70-80%, or 80-90% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 30-40%, preferably 40-50%, more preferably 50-60%, 60-70%, 70-80%, or 80-90%

homology and share a common functional activity are defined herein as sufficiently homologous.

Accordingly, another embodiment of the invention features isolated CSATP-1 proteins and polypeptides having a CSATP-1 activity. Preferred proteins are CSATP-1 proteins having at least one CSATP-1 unique N-terminal domain and at least one PTPase active domain. Other preferred proteins are CSATP-1 proteins having at least a CSATP-1 unique N-terminal domain, at least one phosphatase active domain, and at least one phosphatase extended catalytic active domain. Additional preferred proteins are CSATP-1 proteins having at least a CSATP-1 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, and at least one phosphatase catalytic active domain. Yet other preferred proteins are CSATP-1 proteins having at least a CSATP-1 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, at least one phosphatase catalytic active domain, and at least one phosphatase catalytic core active domain. Other preferred proteins are CSATP-1 proteins having at least a CSATP-1 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, and at least one phosphatase catalytic active domain, at least one phosphatase catalytic core active domain, at least one phosphatase catalytic invariant core active domain. Further preferred proteins are CSATP-1 proteins having at least a CSATP-1 unique N-terminal domain, at least one phosphatase active domain, and at least one intradomain disulfide bond consensus sequence.

The nucleotide sequence of the isolated human CSATP-1 cDNA and the predicted amino acid sequence of the human CSPATP-1 polypeptide are shown in Figure 1 and in SEQ ID NOS:1, 3 and 2, respectively. A plasmid containing the nucleotide sequence encoding human CSATP-1 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on \_\_\_\_\_ and assigned Accession Number \_\_\_\_\_. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely

as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The CSAPTP-1 gene, which is approximately 1315 nucleotides in length, encodes a protein having a molecular weight of approximately 19 kD and which is  
5 approximately 173 amino acid residues in length. CSAPTP-1 message was detected in human heart and skeletal, in all rat tissues but predominantly in rat skeletal, heart, placenta, lung and brain.

In a preferred embodiment, CSAPTP-1 proteins of the invention have an amino acid sequence of about 50-100, more preferably about 100-150, and even more  
10 preferably about 150-200 or 173 amino acid residues in length.

#### B. The CSAPTP-2 Nucleic Acid and Protein Molecules

In another embodiment, isolated proteins of the present invention, preferably CSAPTP-2 proteins, are identified based on the presence of a CSAPTP-2 unique N-  
15 terminal domain and at least one phosphatase active domain as defined herein. As used herein, a "CSAPTP-2 unique N-terminal domain" includes a protein domain which is at least 110-130 amino acid residues in length, preferably at least 115-145 amino acid residues in length, more preferably at least 120-140, or at least 125-135, or preferably 131 amino acid residues in length. In another embodiment, a CSAPTP-2 unique N-  
20 terminal domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a CSAPTP-2 unique N-terminal domain of a human CSAPTP-2 sequence set forth in SEQ ID NO:5 (e.g., amino acid residues 1-131 of the amino acid sequence as set forth in SEQ ID NO:5). As further defined herein, a CSAPTP-2 unique N-terminal domain of a CSAPTP-2 protein  
25 family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-2 protein family. Preferably, a "CSAPTP-2 unique N-terminal domain" has amino acid residues 1-131 of SEQ ID NO:5.

In yet another embodiment, the isolated CSAPTP-2 proteins are identified based  
30 on the presence of a CSAPTP-2 unique N-terminal domain, at least one phosphatase active domain, and at least one phosphatase extended catalytic active domain. In yet

another embodiment, the isolated CSAPTP-2 proteins are identified based on the presence of a CSAPTP-2 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain and at least one phosphatase catalytic active domain. In yet another embodiment, the isolated CSAPTP-  
5 2 proteins are identified based on the presence of a CSAPTP-2 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, at least one phosphatase catalytic active domain and at least one phosphatase catalytic core active domain. In yet another embodiment, the isolated CSAPTP-2 proteins are identified based on the presence of a CSAPTP-2 unique N-  
10 terminal domain and at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, at least one phosphatase catalytic active domain, at least one phosphatase catalytic core active domain, at least one phosphatase catalytic invariant core active domain, each of the above domains are described herein.

In a preferred embodiment, a CSAPTP-2 unique N-terminal domain includes  
15 amino acids 1-131. In another embodiment, a CSAPTP-2 phosphatase active domain includes amino acid residues 79-263. In another embodiment, a CSAPTP-2 phosphatase extended catalytic active domain includes amino acid residues 105-125. In another embodiment, a CSAPTP-2 phosphatase catalytic active domain includes amino acid residues 107-116. In another embodiment, a CSAPTP-2 phosphatase catalytic core  
20 active domain includes amino acid residues 108-116. In another embodiment, a CSAPTP-2 phosphatase catalytic invariant core active domain includes amino acid residues 109-115.

In another embodiment, CSAPTP-2 family members include at least 1, 2, 3 or more Protein kinase C (PKC) phosphorylation sites. PKC phosphorylation sites can be  
25 found at least at residues 11-13, 220-222 and 223-225 of SEQ ID NO:5. CSAPTP-2 family members can further include at least 1, 2, 3, 4 or more Casein kinase II phosphorylation sites. Casein kinase II phosphorylation sites can be found at least at residues 89-92, 131-134, 165-168 and 257-260 of SEQ ID NO:5. CSAPTP-2 family members can further include at least one amidation site. Amidation sites can be found at  
30 least at residues 176-179 of SEQ ID NO:5. CSAPTP-2 family members can further include at least 1 or more N-glycosylation sites. N-glycosylation sites can be found at

least at residues 50-53 of SEQ ID NO:5. CSATP-2 family members can further include at least 1, 2, 3, 4, 5 or more N-myristoylation sites. N-myristoylation sites can be found at least at residues 25-30, 58-63, 112-117, 160-165 and 246-251 of SEQ ID NO:5. CSATP-2 family members can further include at least 1, 2 or more helix-loop-helix DNA-binding domains. Helix-loop-helix DNA-binding domains can be found at least at residues 31-43 of SEQ ID NO:5.

Isolated proteins of the present invention, preferably CSATP-2 proteins, have an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:5 or are encoded by a nucleotide sequence which includes a nucleotide sequence sufficiently homologous to SEQ ID NO:4, SEQ ID NO:6. As used herein, the term "sufficiently homologous" includes a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30-40% homology, preferably 40-50% homology, more preferably 50-60%, and even more preferably 60-70%, 70-80%, or 80-90% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 30-40%, preferably 40-50%, more preferably 50-60%, 60-70%, 70-80%, or 80-90% homology and share a common functional activity are defined herein as sufficiently homologous.

Accordingly, another embodiment of the invention features isolated CSATP-2 proteins and polypeptides having a CSATP-2 activity as defined herein.

The nucleotide sequence of the isolated human CSATP-2 cDNA and the predicted amino acid sequence of the human CSATP-2 polypeptide are shown in Figure 2 and in SEQ ID NOS:4,6 and 5, respectively. A plasmid containing the nucleotide sequence encoding human CSATP-2 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209,

on \_\_\_\_\_ and assigned Accession Number \_\_\_\_\_. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is  
5 required under 35 U.S.C. §112.

The CSAPTP-2 gene, which is approximately 1016 nucleotides in length, encodes a protein having a molecular weight of approximately 29 kD and which is approximately 263 amino acid residues in length. CSAPTP-2 message was detected in human with highest expression in skeletal muscle with some expression in heart and  
10 brain muscle, while in the rat expression was mainly found in skeletal muscle.

In a preferred embodiment, CSAPTP-2 proteins of the invention have an amino acid sequence of about 50-100, more preferably about 100-200, more preferably about 200-250, and even more preferably about 250-300 or 263 amino acid residues in length.

15 C. The CSAPTP-3 Nucleic Acid and Protein Molecules

In another embodiment, the isolated proteins of present invention, preferably CSAPTP-3 proteins, are identified based on the presence of a CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain. As used herein, a "CSAPTP-3 unique N-terminal domain" includes a protein domain which is at least 100-  
20 110 amino acid residues in length, preferably at least 95-115 amino acid residues in length, more preferably at least 90-120, or at least 85-125, or preferably 104 amino acid residues in length. In another embodiment, a CSAPTP-3 unique N-terminal domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a CSAPTP-3 unique N-terminal domain of a  
25 human CSAPTP-3 sequence set forth in SEQ ID NO:8 (e.g., amino acid residues 1-104 of the amino acid sequence as set forth in SEQ ID NO:8). As further defined herein, a CSAPTP-3 unique N-terminal domain of a CSAPTP-3 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-3 protein family. Preferably, a "CSAPTP-3  
30 unique N-terminal domain" has amino acid residues 1-104 of SEQ ID NO:8

Accordingly, another embodiment of the invention features isolated CSAPTP-3 proteins and polypeptides having a CSAPK-3 activity. Preferred proteins are CSAPTP-3 proteins having at least a CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain and, optionally, at least one intradomain disulfide bond consensus sequence. Other preferred proteins are CSAPTP-3 proteins having at least a CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain, and at least one phosphatase extended catalytic active domain. Yet other preferred proteins are CSAPK-3 proteins having at least a CSAPTP-3 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, and at least one phosphatase catalytic active domain. Additionally, preferred proteins are CSAPTP-3 proteins having at least a CSAPTP-3 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, at least one phosphatase catalytic active domain, and at least one phosphatase catalytic core active domain. Yet other preferred proteins are CSAPTP-3 proteins having at least a CSAPTP-3 unique N-terminal domain, at least one phosphatase extended catalytic active domain, at least one phosphatase catalytic active domain, at least one phosphatase catalytic core active domain, and at least one phosphatase catalytic invariant core active domain.

The CSAPTP-3 unique N-terminal domain, the intradomain disulfide bond consensus sequence, the phosphatase active domain, the phosphatase extended catalytic active domain, the phosphatase catalytic active domain, the phosphatase catalytic core active domain, and the phosphatase catalytic invariant core active domain are described herein. In a preferred embodiment, a CSAPTP-3 unique N-terminal domain includes amino acid residues 1-104. In another embodiment, a CSAPTP-3 disulfide bond consensus sequence includes amino acid residues 131-137. In another embodiment, a CSAPTP-3 phosphatase active domain includes amino acid residues 105-209. In another embodiment, a CSAPTP-3 phosphatase extended catalytic active domain includes amino acid residues 134-154. In another embodiment, a CSAPTP-3 phosphatase catalytic active domain includes amino acid residues 136-145. In another embodiment, a CSAPTP-3 phosphatase catalytic core active domain includes amino acid

residues 137-145. In another embodiment, a CSAPTP-3 phosphatase catalytic invariant core active domain includes amino acid residues 138-144.

In another embodiment, CSAPTP-3 family members include at least 1, 2, 3 or more Protein kinase C (PKC) phosphorylation sites. PKC phosphorylation sites can be found at least at residues 22-24, 169-171 and 190-192 of SEQ ID NO:8. CSAPTP-3 family members can further include at least 1, 2, 3 or more Casein kinase II phosphorylation sites. Casein kinase II phosphorylation sites can be found at least at residues 76-79, 89-92 and 104-107 of SEQ ID NO:8. CSAPTP-3 family members can further include at least one cAMP and cGMP dependent protein kinase phosphorylation site. cAMP and cGMP dependent protein kinase phosphorylation sites can be found at least at residues 24-27 of SEQ ID NO:8. CSAPTP-3 family members can further include at least one or more N-glycosylation sites. N-glycosylation sites can be found at least at residues 88-91 of SEQ ID NO:8. CSAPTP-3 family members can further include at least 1, 2, 3, 4 or more N-myristoylation sites. N-myristoylation sites can be found at least at residues 19-24, 41-46 and 120-125 of SEQ ID NO:8. CSAPTP-3 family members can further include at least 1, 2 or more transcription factor TFIIB repeats. Transcription factor TFIIB repeats can be found at least at residues 108-179 of SEQ ID NO:8.

Isolated proteins of the present invention, preferably CSAPTP-3 proteins, have an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:8 or are encoded by a nucleotide sequence which includes a nucleotide sequence sufficiently homologous to SEQ ID NO:7, SEQ ID NO:9. As used herein, the term "sufficiently homologous" includes a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30-40% homology, preferably 40-50% homology, more preferably 50-60%, and even more preferably 60-70%, 70-80%, or 80-90% homology across the amino acid sequences of the domains and contain at least one



and preferably two structural domains or motifs, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 30-40%, preferably 40-50%, more preferably 50-60%, 60-70%, 70-80%, or 80-90% homology and share a common functional activity are defined herein as sufficiently  
5 homologous.

The nucleotide sequence of the isolated human CSATP-3 cDNA and the predicted amino acid sequence of the human CSPATP-3 polypeptide are shown in Figure 3 and in SEQ ID NOS:7,9 and 8, respectively. A plasmid containing the nucleotide sequence encoding human CSATP-3 was deposited with American Type  
10 Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on \_\_\_\_\_ and assigned Accession Number \_\_\_\_\_. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is  
15 required under 35 U.S.C. §112.

The CSATP-3 gene, which is approximately 814 nucleotides in length, encodes a protein having a molecular weight of approximately 23 kD and which is approximately 209 amino acid residues in length. CSATP-3 message was detected in human in highest in heart and skeletal and also found in brain, placenta, kidney and pancreas.

20 In a preferred embodiment, CSATP-3 proteins of the invention have an amino acid sequence of about 50-100, more preferably about 100-200, and even more preferably about 200-250 or 209 amino acid residues in length.

#### D. The CSATP-4 Nucleic Acid and Protein Molecules

25 In another embodiment, the isolated proteins of present invention, preferably CSATP-4 proteins, are identified based on the presence of a CSATP-4 unique N-terminal domain and at least one phosphatase active domain. As used herein, a "CSATP-4 unique N-terminal domain" includes a protein domain which is at least 100-200 amino acid residues in length, preferably at least 110-190 amino acid residues in  
30 length, more preferably at least 120-180, or at least 130-170, or preferably about 158 amino acid residues in length. In another embodiment, a CSATP-4 unique N-terminal

domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a CSAPTP-4 unique N-terminal domain of a human CSAPTP-4 sequence set forth in SEQ ID NO:11 (e.g., amino acid residues 1-158 of the amino acid sequence as set forth in SEQ ID NO:11). As further defined

5 herein, a CSAPTP-4 unique N-terminal domain of a CSAPTP-4 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-4 protein. Preferably, a "CSAPTP-4 unique N-terminal domain" has amino acid residues 1-158 of SEQ ID NO:11

Accordingly, another embodiment of the invention features isolated CSAPTP-4

10 proteins and polypeptides having a CSAPK-4 activity. Preferred proteins are CSAPTP-4 proteins having at least a CSAPTP-4 unique N-terminal domain and at least one phosphatase active domain. Other preferred proteins are CSAPTP-4 proteins having at least a CSAPTP-4 unique N-terminal domain and at least one phosphatase active domain, and at least one phosphatase extended catalytic active domain. Yet other

15 preferred proteins are CSAPK-4 proteins having at least a CSAPTP-4 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, and at least one phosphatase catalytic active domain. Additionally, preferred proteins are CSAPTP-4 proteins having at least a CSAPTP-4 unique N-terminal domain, at least one phosphatase active domain, at least one

20 phosphatase extended catalytic active domain, at least one phosphatase catalytic active domain, and at least one phosphatase catalytic core active domain. Yet other preferred proteins are CSAPTP-4 proteins having at least a CSAPTP-4 unique N-terminal domain, at least one phosphatase extended catalytic active domain, at least one phosphatase catalytic active domain, at least one phosphatase catalytic core active domain, and at

25 least one phosphatase catalytic invariant core active domain.

The CSAPTP-4 unique N-terminal domain, the phosphatase active domain, the phosphatase extended catalytic active domain, the phosphatase catalytic active domain, the phosphatase catalytic core active domain, and the phosphatase catalytic invariant core active domain are described herein. In one embodiment, a CSAPTP-4 phosphatase

30 active domain includes amino acid residues 68-221 of SEQ ID NO:11. In another embodiment, a CSAPTP-4 phosphatase extended catalytic active domain includes amino

acid residues 159-171 of SEQ ID NO:11. In another embodiment, a CSAPTP-4 phosphatase catalytic active domain includes amino acid residues 159-168 of SEQ ID NO:11. In another embodiment, a CSAPTP-4 phosphatase catalytic core active domain includes amino acid residues 160-168 of SEQ ID NO:11. In another embodiment, a CSAPTP-3 phosphatase catalytic invariant core active domain includes amino acid residues 161-167 of SEQ ID NO:11.

In another embodiment, CSAPTP-4 family members include at least one Protein kinase C (PKC) phosphorylation site. PKC phosphorylation sites can be found at least at residues 218-220 of SEQ ID NO:11. CSAPTP-4 family members can further include at least one Casein kinase II phosphorylation site. Casein kinase II phosphorylation sites can be found at least at residues 183-186 of SEQ ID NO:11. CSAPTP-4 family members can further include at least one N-glycosylation site. N-glycosylation sites can be found at least at residues 181-184 of SEQ ID NO:11. CSAPTP-4 family members can further include at least one N-myristoylation site. N-myristoylation sites can be found at least at residues 39-44 and 164-169 of SEQ ID NO:11.

Isolated proteins of the present invention, preferably CSAPTP-4 proteins, have an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:11 or are encoded by a nucleotide sequence which includes a nucleotide sequence sufficiently homologous to SEQ ID NO:10, SEQ ID NO:12. As used herein, the term "sufficiently homologous" includes a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30-40% homology, preferably 40-50% homology, more preferably 50-60%, and even more preferably 60-70%, 70-80%, or 80-90% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 30-40%, preferably 40-50%, more preferably 50-60%, 60-70%, 70-80%, or 80-90%

homology and share a common functional activity are defined herein as sufficiently homologous.

The nucleotide sequence of the isolated human CSATP-4 cDNA and the predicted amino acid sequence of the human CSPATP-4 polypeptide are shown in Figure 9 and in SEQ ID NOS:10, 12 and 11, respectively. A plasmid containing the nucleotide sequence encoding human CSATP-3 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on \_\_\_\_\_ and assigned Accession Number \_\_\_\_\_. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The CSATP-4 gene, which is approximately 928 nucleotides in length, encodes a protein having a molecular weight of approximately 24 kD and which is approximately 221 amino acid residues in length. CSATP-4 message was detected in human heart and skeletal muscle.

In another preferred embodiment, CSATP-4 nucleic acids of the invention include at least 100 consecutive nucleotides, more preferably at least 200 consecutive nucleotides, more preferably at least 400 consecutive nucleotides, more preferably at least 600 consecutive nucleotides, more preferably at least 800 consecutive nucleotides, more preferably at least 900 consecutive nucleotides of the nucleotide sequence shown SEQ ID NO:10 or 12, or a complement thereof.

In another preferred embodiment, CSATP-4 proteins of the invention include at least 50 consecutive amino acids, more preferably at least 100 consecutive amino acids, more preferably at least 150 consecutive amino acids, more preferably at least 200 consecutive amino acids of the amino acid sequence shown SEQ ID NO:11.

#### E. CSATP Activities

As used interchangeably herein, a "CSATP activity", "biological activity of CSATP" or "functional activity of CSATP", includes an activity exerted by a CSATP protein, polypeptide or nucleic acid molecule as determined *in vivo*, *in vitro*, or

*in situ*, according to standard techniques. In one embodiment, a CSAPTP activity is a direct activity, such as an association with a CSAPTP-target molecule. As used herein, a "target molecule" is a molecule with which a CSAPTP protein binds or interacts in nature, such that CSAPTP-mediated function is achieved. A CSAPTP target molecule  
5 can be a CSAPTP protein or polypeptide of the present invention or a non-CSAPTP molecule. For example, a CSAPTP target molecule can be a non-CSAPTP protein molecule. Alternatively, a CSAPTP activity is an indirect activity, such as an activity mediated by interaction of the CSAPTP protein with a CSAPTP target molecule such that the target molecule modulates a downstream cellular activity (e.g., interaction of an  
10 CSAPTP molecule with a CSAPTP target molecule can modulate the activity of that target molecule on an immune cell).

In a preferred embodiment, a CSAPTP activity is at least one or more of the following activities: (i) interaction of a CSAPTP protein with a CSAPTP target molecule; (ii) interaction of a CSAPTP protein with a CSAPTP target molecule,  
15 wherein the CSAPTP target is a ligand, e.g., phosphorylated amino acid residue of a phosphorylated protein (e.g., a phosphatase, for example, a cell cycle regulatory phosphatase, e.g., Cdc25A phosphatase, a cell cycle phosphatase which regulates the G1/S-phase transition, a CSAPTPase), a kinase (e.g., Mitogen Activating Protein (MAP) kinase, or a Cardiovascular associated Protein Tyrosine Kinase (CSAPTK)); (iii)  
20 interaction of a CSAPTP protein with a CSAPTP target molecule, wherein the CSAPTP target is a receptor, e.g., insulin receptor, insulin receptor substrate 1; (iv) interaction of a CSAPTP protein with a CSAPTP target molecule, wherein the CSAPTP target is a viral protein, e.g., *vaccinia* viral transcription-mediating proteins, Myxoma viral proteins, Shope Fibroma viral proteins, *Leishmania donovani*, *Trypanosoma brucei* and  
25 *Trypanosoma cruzi* viral proteins.

In yet another preferred embodiment, a CSAPTP activity is at least one or more of the following activities: (1) regulation of cell cycle, e.g., dephosphorylation of phosphorylated proteins involved in the cell cycle, e.g., dephosphorylation of phosphorylated proteins involved in the cell cycle, e.g., proliferation-mediating proteins,  
30 e.g., Cdc25A, a cell cycle phosphatase which regulates the G1/S-phase transition, e.g., anti-proliferative proteins, either *in vitro*, *in vivo* or *in situ*; (2) mediation of the viral

pathogenicity, e.g., viral phosphatase mediated dephosphorylation of host phosphorylated proteins, for example, viral induced disease, e.g., *Yersinia* pathogenesis, for example, *Yersinia pestis* (Bubonic Plague), e.g., viral phosphatase mediated dephosphorylation of host anti-proliferative phosphorylated proteins, for example, viral  
5 induced proliferative diseases, e.g., viral induced cancers, either *in vitro*, *in vivo* or *in situ*; (3) regulation of the phosphorylation state of receptors, e.g., insulin receptor, e.g., insulin receptor substrate 1, either *in vitro*, *in vivo* or *in situ*.

Various aspects of the invention are described in further detail in the following  
10 subsections:

#### I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode CSAPTP proteins or biologically active portions thereof, as well as nucleic acid  
15 fragments sufficient for use as hybridization probes to identify CSAPTP-encoding nucleic acids (e.g., CSAPTP mRNA) and fragments for use as PCR primers for the amplification or mutation of CSAPTP nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated  
20 using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e.,  
25 sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated CSAPTP nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived.  
30 Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by

recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. For example, using all or portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, as a hybridization probe, CSAPTP nucleic acid molecules can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_ can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to CSAPTP nucleotide sequences can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:10. The sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:10, corresponds to the human CSAPTP-1, CSAPTP-2 CSAPTP-3 and CSAPTP-4 cDNAs, respectively. These cDNAs comprise sequence encoding the human CSAPTP-1, CSAPTP-2, CSAPTP-3 and CSAPTP-4 proteins, i.e., "the coding region", from nucleotides 247-767, 1-789, 1-627 and 1-663 respectively, as well as 5' untranslated and 3' untranslated sequences. Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:10 (e.g., nucleotides 247-767, 1-789, 1-627 and 1-663, respectively), corresponding to SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, and SEQ ID NO:12, respectively).

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO. 12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_ or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 12, respectively, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 12, respectively, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, thereby forming a stable duplex.



In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least 30, 31, 32-35%, preferably about 35-36%, 36-40%, more preferably at least 40-43%, 43-45%, more preferably at least 45-50%, and even more preferably at least 50-55%, 55-57%, 57-60%, 5 60-65%, 65-70%, 70-75%, 75-80%, 80-85%, 85-90%, or 90-95% or more homologous to the nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, or a portion of any of these 10 nucleotide sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence (e.g., to the entire length of the nucleotide sequence) of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 12, or the nucleotide sequence of the DNA insert 15 of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_. for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of a CSAPTP protein. The nucleotide sequence determined from the cloning of the CSAPTP gene allows for the generation of probes and primers designed for use in identifying and/or cloning other CSAPTP family 20 members, as well as CSAPTP homologues from other species. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, SEQ ID 25 NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, of an anti-sense sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 12, or the nucleotide sequence of the DNA insert 30 of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, or of a naturally occurring mutant of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ

ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_.

5 In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is about 100, preferably 100-200, preferably 200-300, more preferably 300-400, more preferably 400-500, and even more preferably 500-516 nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_.

10 In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is about 450, preferably 450-550, more preferably 550-650, more preferably 650-750, and even more preferably 750-789 nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA  
15 insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_.

In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is about 300, preferably 300-400, more preferably 400-500, more preferably 500-600, and even more preferably 600-627 nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic  
20 acid molecule of SEQ ID NO:7, SEQ ID NO:9, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_.

In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is about 200, preferably 200-300, more preferably 300-400, more preferably 400-500, and even more preferably 500-600 or  
25 more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_.

Probes based on the CSAPTP nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In  
30 preferred embodiments, the probe further comprises a label group attached thereto, e.g., the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme

co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a CSAPTP protein, such as by measuring a level of a CSAPTP-encoding nucleic acid in a sample of cells from a subject e.g., detecting CSAPTP mRNA levels or determining whether a genomic CSAPTP gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of a CSAPTP protein" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, which encodes a polypeptide having a CSAPTP biological activity (the biological activities of the CSAPTP proteins have previously been described), expressing the encoded portion of the CSAPTP protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the CSAPTP protein.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, due to degeneracy of the genetic code and thus encode the same CSAPTP proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO: 11.

In addition to the CSAPTP nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the

amino acid sequences of the CSAPTP proteins may exist within a population (e.g., the human population). Such genetic polymorphism in the CSAPTP genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open  
5 reading frame encoding a CSAPTP protein, preferably a mammalian CSAPTP protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of a CSAPTP gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in CSAPTP genes that are the result of natural allelic variation and that do not alter the functional activity of a CSAPTP protein are intended  
10 to be within the scope of the invention.

Moreover, nucleic acid molecules encoding other CSAPTP family members, and thus which have a nucleotide sequence which differs from the CSAPTP sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID  
15 NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_ are intended to be within the scope of the invention. For example, a CSAPTP cDNA can be identified based on the nucleotide sequence of human CSAPTP. Moreover, nucleic acid molecules encoding CSAPTP proteins from different species, and thus which have a nucleotide sequence which differs from the CSAPTP sequences of SEQ ID NO:1, SEQ  
20 ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_ are intended to be within the scope of the invention. For example, an mouse CSAPTP cDNA can be identified based on the nucleotide sequence of a human CSAPTP.

25 Nucleic acid molecules corresponding to natural allelic variants and homologues of the CSAPTP cDNAs of the invention can be isolated based on their homology to the CSAPTP nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_. In other embodiment, the nucleic acid is at least 30, 50, 100, 250, 500, 750, 1000, 1500 nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more homologous to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least 70%, more preferably at least 80%, even more preferably at least 85% or 90% homologous to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, or SEQ ID NO:12, corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule includes an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

In addition to naturally-occurring allelic variants of the CSAPTP sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, thereby leading to changes in the amino acid sequence of the encoded CSAPTP proteins, without altering the functional

ability of the CSAPTP proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_, \_\_\_\_, or \_\_\_\_\_. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of CSAPTP (e.g., the sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the CSAPTP proteins of the present invention, are predicted to be particularly unamenable to alteration (e.g., the ten conserved cysteines involved in forming disulfide linkages or the conserved histidine, aspartate, or serine of the active enzymatic site). Moreover, amino acid residues that are defined by the CSAPTP intradomain disulfide bond consensus sequence, the phosphatase active domain, the phosphatase extended catalytic active domain, the phosphatase catalytic active domain, the phosphatase catalytic core active domain, and the phosphatase catalytic invariant core active domain are signature sequences and are therefore particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the CSAPTP proteins of the present invention and other members of the Protein Tyrosine Phosphatase superfamily or protein families containing tyrosine, serine or threonine phosphatase activity are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding CSAPTP proteins that contain changes in amino acid residues that are not essential for activity. Such CSAPTP proteins differ in amino acid sequence from SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11 yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least 10%, 15%, 20%, 23%, 24%, 25%, 30%, 35%, 40%, 42%, 45%, 50%, 55%, 57%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11. Preferably, the protein encoded by the nucleic acid molecule is at least 65-70% homologous to SEQ ID

NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, more preferably at least 75-80% homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, even more preferably at least 85-90% homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, and most preferably at least 95% homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11 (e.g., the entire amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11).

An isolated nucleic acid molecule encoding a CSAPTP protein homologous to the protein of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_ by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a CSAPTP protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly

along all or part of a CSAPTP coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for CSAPTP biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

In a preferred embodiment, a mutant CSAPTP protein can be assayed for the ability to (1) regulate the cell cycle, e.g., dephosphorylation of phosphorylated proteins involved in the cell cycle, e.g., dephosphorylation of phosphorylated proteins involved in the cell cycle, e.g., proliferation-mediating proteins, e.g., Cdc25A, a cell cycle phosphatase which regulates the G1/S-phase transition, e.g., anti-proliferative proteins, either *in vitro*, *in vivo* or *in situ*; (2) mediation of the viral pathogenicity, e.g., viral phosphatase mediated dephosphorylation of host phosphorylated proteins, for example, viral induced disease, e.g., *Yersinia* pathogenesis, for example, *Yersinia pestis* (Bubonic Plague), e.g., viral phosphatase mediated dephosphorylation of host anti-proliferative phosphorylated proteins, for example, viral induced proliferative diseases, e.g., viral induced cancers, either *in vitro*, *in vivo* or *in situ*; (3) regulation of the phosphorylation state of receptors, e.g., insulin receptor, e.g., insulin receptor substrate 1, either *in vitro*, *in vivo* or *in situ*.

In addition to the nucleic acid molecules encoding CSAPTP proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire CSAPTP coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding CSAPTP. The term "coding region" includes the region of the nucleotide sequence comprising codons which are translated into amino acid residues (e.g., the coding region of human CSAPTP corresponds to ). In



another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding CSAPTP. The term "noncoding region" includes 5' and 3' sequences which flank the coding region that are not translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions).

- 5        Given the coding strand sequences encoding CSAPTP disclosed herein (e.g., SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of CSAPTP mRNA, but more preferably is an oligonucleotide which is antisense
- 10       to only a portion of the coding or noncoding region of CSAPTP mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of CSAPTP mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and
- 15       enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate
- 20       derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-
- 25       galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine,
- 30       uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid

methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a CSATP protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An  $\alpha$ -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes  
5 (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave CSAPTP mRNA transcripts to thereby inhibit translation of CSAPTP mRNA. A ribozyme having specificity for a CSAPTP-encoding nucleic acid can be designed based upon the nucleotide sequence of a CSAPTP cDNA disclosed herein (i.e., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7,  
10 SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a CSAPTP-encoding mRNA. See, e.g., Cech *et al.* U.S.  
15 Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, CSAPTP mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, CSAPTP gene expression can be inhibited by targeting nucleotide  
20 sequences complementary to the regulatory region of the CSAPTP (e.g., the CSAPTP promoter and/or enhancers) to form triple helical structures that prevent transcription of the CSAPTP gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

25 In yet another embodiment, the CSAPTP nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal  
30 Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate

backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis  
5 protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* PNAS 93: 14670-675.

PNAs of CSAPTP nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing  
10 transcription or translation arrest or inhibiting replication. PNAs of CSAPTP nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*;  
15 Perry-O'Keefe *supra*).

In another embodiment, PNAs of CSAPTP can be modified, (e.g., to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of CSAPTP  
20 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (e.g., RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of  
25 bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-  
30 thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then

coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

- 5 In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. US.* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810, published December 15, 1988) or the blood-brain
- 10 barrier (see, e.g., PCT Publication No. W089/10134, published April 25, 1988). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol *et al.* (1988) *BioTechniques* 6:958-976) or intercalating agents. (See, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking
- 15 agent, transport agent, or hybridization-triggered cleavage agent).

## II. Isolated CSAPTP Proteins and Anti-CSAPTP Antibodies

- One aspect of the invention pertains to isolated CSAPTP proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as
- 20 immunogens to raise anti-CSAPTP antibodies. In one embodiment, native CSAPTP proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, CSAPTP proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a CSAPTP protein or polypeptide can be synthesized
- 25 chemically using standard peptide synthesis techniques.

- An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the CSAPTP protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language
- 30 "substantially free of cellular material" includes preparations of CSAPTP protein in which the protein is separated from cellular components of the cells from which it is

isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of CSAPTP protein having less than about 30% (by dry weight) of non-CSAPTP protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-CSAPTP protein, 5 still more preferably less than about 10% of non-CSAPTP protein, and most preferably less than about 5% non-CSAPTP protein. When the CSAPTP protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the 10 protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of CSAPTP protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or 15 other chemicals" includes preparations of CSAPTP protein having less than about 30% (by dry weight) of chemical precursors or non-CSAPTP chemicals, more preferably less than about 20% chemical precursors or non-CSAPTP chemicals, still more preferably less than about 10% chemical precursors or non-CSAPTP chemicals, and most preferably less than about 5% chemical precursors or non-CSAPTP chemicals.

20 Biologically active portions of a CSAPTP protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the CSAPTP protein, e.g., the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11, which include less amino acids than the full length CSAPTP proteins, and exhibit at least one activity of a CSAPTP protein. 25 Typically, biologically active portions comprise a domain or motif with at least one activity of the CSAPTP protein. A biologically active portion of a CSAPTP protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

It is to be understood that a preferred biologically active portion of a CSAPTP protein of the present invention may contain at least one of the above-identified 30 structural domains. A more preferred biologically active portion of a CSAPTP protein may contain at least two of the above-identified structural domains. Moreover, other

biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native CSAPTP protein.

In a preferred embodiment, the CSAPTP protein has an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11. In other embodiments, the CSAPTP protein is substantially homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11 and retains the functional activity of the protein of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above.

Accordingly, in another embodiment, the CSAPTP-1 protein is a protein which comprises an amino acid sequence at least 79% homologous to the amino acid sequence of SEQ ID NO:2, and retains the functional activity of the CSAPTP-1 proteins of SEQ ID NO:2. Preferably, the protein is at least 30-35% homologous to SEQ ID NO:2, more preferably at least 35-40% homologous to SEQ ID NO:2, even more preferably at least 40-45% homologous to SEQ ID NO:2, and even more preferably at least 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-79%, 79-80%, 80-85%, 85-90%, or 90-95% or more homologous to SEQ ID NO:2.

Accordingly, in another embodiment, the CSAPTP-2 protein is a protein which comprises an amino acid sequence at least 23% homologous to the amino acid sequence of SEQ ID NO:5 and retains the functional activity of the CSAPTP-2 proteins of SEQ ID NO:5. Preferably, the protein is at least 10%-15% homologous to SEQ ID NO:5, more preferably at least 15-20%, more preferably at least 20-23%, more preferably at least 23-25%, more preferably at least 25-30%, more preferably at least 30-35%, more preferably at least 35-40% homologous to SEQ ID NO:5, even more preferably at least 40-45% homologous to SEQ ID NO:5, and even more preferably at least 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-80%, 80-85%, 85-90%, or 90-95% or more homologous to SEQ ID NO:5.

Accordingly, in another embodiment, the CSAPTP-3 protein is a protein which comprises an amino acid sequence at least 24% homologous to the amino acid sequence of SEQ ID NO:8 and retains the functional activity of the CSAPTP-3 proteins of SEQ

ID NO:8. Preferably, the protein is at least 10-15%, 15-20%, 20-24%, 24-25%, 25-30%, 30-35% homologous to SEQ ID NO:8, more preferably at least 35-40% homologous to SEQ ID NO:8, even more preferably at least 40-45% homologous to SEQ ID NO:8, and even more preferably at least 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-80%, 80-85%, 85-90%, or 90-95% or more homologous to SEQ ID NO:8.

Accordingly, in another embodiment, the CSAPTP-4 protein is a protein which comprises an amino acid sequence at least about 42% or more homologous to the amino acid sequence of SEQ ID NO:11, and retains the functional activity of the CSAPTP-4 proteins of SEQ ID NO:11. Preferably, the protein is at least about 10-15% to SEQ ID NO:11, more preferably at least about 15-20% to SEQ ID NO:11, more preferably at least about 20-25% to SEQ ID NO:11, more preferably at least about 25-30% to SEQ ID NO:11, more preferably at least about 30-35% homologous to SEQ ID NO:11, more preferably at least about 35-40% homologous to SEQ ID NO:11, even more preferably at least about 40-42% homologous to SEQ ID NO:11, and even more preferably at least about 42-45%, 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-80%, 80-85%, 85-90%, or 90-95% or more homologous to SEQ ID NO:11.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (e.g., when aligning a second sequence to the CSAPTP amino acid sequence of SEQ ID NO:2 having 173 amino acid residues, at least 52, preferably at least 70, more preferably at least 87, even more preferably at least 104, and even more preferably at least 121, 138 or 158 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that



position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (*CABIOS*, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to CSAPTP nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to CSAPTP protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25(17):3389-3402.

When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

The invention also provides CSAPTP chimeric or fusion proteins. As used herein, a CSAPTP "chimeric protein" or "fusion protein" comprises a CSAPTP polypeptide operatively linked to a non-CSAPTP polypeptide. A "CSAPTP polypeptide" includes a polypeptide having an amino acid sequence corresponding to CSAPTP, whereas a "non-CSAPTP polypeptide" includes a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the CSAPTP protein, e.g., a protein which is different from the CSAPTP protein and which is derived from the same or a different organism. Within a CSAPTP fusion protein the CSAPTP polypeptide can correspond to all or a portion of a CSAPTP protein. In a preferred embodiment, a CSAPTP fusion protein comprises at least one biologically active portion of a CSAPTP protein. In another preferred embodiment, a CSAPTP fusion protein comprises at least two biologically active portions of a CSAPTP protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the CSAPTP polypeptide and the non-CSAPTP polypeptide are fused in-frame to each other. The non-CSAPTP polypeptide can be fused to the N-terminus or C-terminus of the CSAPTP polypeptide.

For example, in one embodiment, the fusion protein is a GST-CSAPTP fusion protein in which the CSAPTP sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant CSAPTP.

In another embodiment, the fusion protein is a CSAPTP protein containing a heterologous signal sequence at its N-terminus. For example, the native CSAPTP signal sequence can be removed and replaced with a signal sequence from another protein. In certain host cells (e.g., mammalian host cells), expression and/or secretion of CSAPTP can be increased through use of a heterologous signal sequence.

The CSAPTP fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The CSAPTP fusion proteins can be used to affect the bioavailability of a CSAPTP target molecule. Use of

CSAPTP fusion proteins may be useful therapeutically for the treatment of proliferative disorders (e.g., cancer). Moreover, the CSAPTP-fusion proteins of the invention can be used as immunogens to produce anti-CSAPTP antibodies in a subject, to purify CSAPTP ligands and in screening assays to identify molecules which inhibit the  
5 interaction of CSAPTP with a CSAPTP target molecule.

Preferably, a CSAPTP chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended  
10 termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor  
15 primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A CSAPTP-  
20 encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the CSAPTP protein.

The present invention also pertains to variants of the CSAPTP proteins which function as either CSAPTP agonists (mimetics) or as CSAPTP antagonists. Variants of the CSAPTP proteins can be generated by mutagenesis, e.g., discrete point mutation or  
25 truncation of a CSAPTP protein. An agonist of the CSAPTP proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a CSAPTP protein. An antagonist of a CSAPTP protein can inhibit one or more of the activities of the naturally occurring form of the CSAPTP protein by, for example, competitively inhibiting the phosphatase activity of a CSAPTP protein. Thus, specific  
30 biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological

activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the CSAPTP protein.

In one embodiment, variants of a CSAPTP protein which function as either CSAPTP agonists (mimetics) or as CSAPTP antagonists can be identified by screening  
5 combinatorial libraries of mutants, e.g., truncation mutants, of a CSAPTP protein for CSAPTP protein agonist or antagonist activity. In one embodiment, a variegated library of CSAPTP variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of CSAPTP variants can be produced by, for example, enzymatically ligating a mixture of synthetic  
10 oligonucleotides into gene sequences such that a degenerate set of potential CSAPTP sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of CSAPTP sequences therein. There are a variety of methods which can be used to produce libraries of potential CSAPTP variants from a degenerate oligonucleotide sequence. Chemical  
15 synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential CSAPTP sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang, S.A.  
20 (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of a CSAPTP protein coding sequence can be used to generate a variegated population of CSAPTP fragments for screening and subsequent selection of variants of a CSAPTP protein. In one embodiment, a library of  
25 coding sequence fragments can be generated by treating a double stranded PCR fragment of a CSAPTP coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed  
30 duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into

an expression vector. By this method, an expression library can be derived which encodes N-terminal, and internal fragments of various sizes of the CSAPTP protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA  
5 libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of CSAPTP proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the  
10 resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify CSAPTP variants (Arkin and Yourvan  
15 (1992) *PNAS* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

In one embodiment, cell based assays can be exploited to analyze a variegated CSAPTP library. For example, a library of expression vectors can be transfected into a cell line which ordinarily synthesizes and secretes CSAPTP. The transfected cells are then cultured such that CSAPTP and a particular mutant CSAPTP are secreted and the  
20 effect of expression of the mutant on CSAPTP activity in cell supernatants can be detected, e.g., by any of a number of enzymatic assays. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of CSAPTP activity, and the individual clones further characterized.

An isolated CSAPTP protein, or a portion or fragment thereof, can be used as an  
25 immunogen to generate antibodies that bind CSAPTP using standard techniques for polyclonal and monoclonal antibody preparation. A full-length CSAPTP protein can be used or, alternatively, the invention provides antigenic peptide fragments of CSAPTP for use as immunogens. The antigenic peptide of a CSAPTP protein comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5,  
30 SEQ ID NO:8, or SEQ ID NO:11 and encompasses an epitope of CSAPTP such that an antibody raised against the peptide forms a specific immune complex with CSAPTP.

Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of CSAPTP  
5 that are located on the surface of the protein, e.g., hydrophilic regions.

A CSAPTP immunogen typically is used to prepare antibodies by immunizing a suitable subject, (e.g., rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed CSAPTP protein or a chemically synthesized CSAPTP polypeptide. The  
10 preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic CSAPTP preparation induces a polyclonal anti-CSAPTP antibody response.

Accordingly, another aspect of the invention pertains to anti-CSAPTP  
15 antibodies. The term "antibody" as used herein includes immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as CSAPTP. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')<sub>2</sub> fragments which can be generated by treating the  
20 antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind CSAPTP. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, includes a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of CSAPTP. A monoclonal antibody  
25 composition thus typically displays a single binding affinity for a particular CSAPTP protein with which it immunoreacts.

Polyclonal anti-CSAPTP antibodies can be prepared as described above by immunizing a suitable subject with a CSAPTP immunogen. The anti-CSAPTP antibody titer in the immunized subject can be monitored over time by standard techniques, such  
30 as with an enzyme linked immunosorbent assay (ELISA) using immobilized CSAPTP. If desired, the antibody molecules directed against CSAPTP can be isolated from the

mammal (e.g., from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-CSAPTP antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *PNAS* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387-402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a CSAPTP immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds CSAPTP.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-CSAPTP monoclonal antibody (see, e.g., G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of

- myeloma cell lines can be used as a fusion partner according to standard techniques, e.g., the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind CSAPTP, e.g., using a standard ELISA assay.
- Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-CSAPTP antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with CSAPTP to thereby isolate immunoglobulin library members that bind CSAPTP. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *PNAS* 88:7978-7982; and McCafferty *et al.* *Nature* (1990) 348:552-554.



Additionally, recombinant anti-CSAPTP antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *PNAS* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *PNAS* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoevan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

An anti-CSAPTP antibody (e.g., monoclonal antibody) can be used to isolate CSAPTP by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-CSAPTP antibody can facilitate the purification of natural CSAPTP from cells and of recombinantly produced CSAPTP expressed in host cells. Moreover, an anti-CSAPTP antibody can be used to detect CSAPTP protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the CSAPTP protein. Anti-CSAPTP antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic

group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

### III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a CSAPTP protein (or a portion thereof). As used herein, the term "vector" includes a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which includes a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is

operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., CSAPTP proteins, mutant forms of CSAPTP proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of CSAPTP proteins in prokaryotic or eukaryotic cells. For example, CSAPTP proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein;

2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in CSAPTP activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for CSAPTP proteins, for example. In a preferred embodiment, a CSAPTP fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six (6) weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another

strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard

5 DNA synthesis techniques.

In another embodiment, the CSAPTP expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (InVitrogen Corp, San Diego, CA).

Alternatively, CSAPTP proteins can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 15 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY,* 25 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* 30

(1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the  $\alpha$ -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to CSAPTP mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny

of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

- 5           A host cell can be any prokaryotic or eukaryotic cell. For example, a CSAPTP protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

- Vector DNA can be introduced into prokaryotic or eukaryotic cells via  
10   conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or  
15   transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual*, 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

- For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may  
20   integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be  
25   introduced into a host cell on the same vector as that encoding a CSAPTP protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

- A host cell of the invention, such as a prokaryotic or eukaryotic host cell in  
30   culture, can be used to produce (i.e., express) a CSAPTP protein. Accordingly, the invention further provides methods for producing a CSAPTP protein using the host cells

of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding a CSAPTP protein has been introduced) in a suitable medium such that a CSAPTP protein is produced. In another embodiment, the method further comprises isolating a CSAPTP protein from the  
5 medium or the host cell.

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which CSAPTP-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in  
10 which exogenous CSAPTP sequences have been introduced into their genome or homologous recombinant animals in which endogenous CSAPTP sequences have been altered. Such animals are useful for studying the function and/or activity of a CSAPTP and for identifying and/or evaluating modulators of CSAPTP activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a  
15 rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an  
20 encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous CSAPTP gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the  
25 animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing a CSAPTP-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The CSAPTP cDNA sequence of SEQ ID NO:1,  
30 SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:12 can be introduced as a transgene into the genome of a non-



human animal. Alternatively, a nonhuman homologue of a human CSAPTP gene, such as a mouse or rat CSAPTP gene, can be used as a transgene. Alternatively, a CSAPTP gene homologue, such as another CSAPTP family member, can be isolated based on hybridization to the CSAPTP cDNA sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, or \_\_\_\_\_ (described further in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a CSAPTP transgene to direct expression of a CSAPTP protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a CSAPTP transgene in its genome and/or expression of CSAPTP mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a CSAPTP protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a CSAPTP gene into which a deletion, addition or substitution has been introduced to thereby alter, e.g., functionally disrupt, the CSAPTP gene. The CSAPTP gene can be a human gene (e.g., the cDNA of ), but more preferably, is a non-human homologue of a human CSAPTP gene (e.g., a cDNA isolated by stringent hybridization with the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:12). For example, a mouse CSAPTP gene can be used to construct a homologous recombination vector suitable for altering an endogenous CSAPTP gene in the mouse

genome. In a preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous CSAPTP gene is functionally disrupted (i.e., no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous

5 CSAPTP gene is mutated or otherwise altered but still encodes functional protein (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous CSAPTP protein). In the homologous recombination vector, the altered portion of the CSAPTP gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the CSAPTP gene to allow for homologous recombination to occur between

10 the exogenous CSAPTP gene carried by the vector and an endogenous CSAPTP gene in an embryonic stem cell. The additional flanking CSAPTP nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (see e.g., Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a

15 description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced CSAPTP gene has homologously recombined with the endogenous CSAPTP gene are selected (see e.g., Li, E. *et al.* (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (see e.g.,

20 Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously

25 recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

In another embodiment, transgenic non-humans animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, e.g., Lakso *et al.* (1992) *PNAS* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813. In brief, a cell, e.g., a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G<sub>0</sub> phase. Alternatively, a cell, e.g., an embryonic stem cell, from the inner cell mass of a developing embryo can be transformed with a preferred transgene. Alternatively, a cell, e.g., a somatic cell, from cell culture line can be transformed with a preferred transgene and induced to exit the growth cycle and enter G<sub>0</sub> phase. The cell can then be fused, e.g., through the use of electrical pulses, to an enucleated mammalian oocyte. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the nuclear donor cell, e.g., the somatic cell, is isolated.

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#### IV. Pharmaceutical Compositions

The CSAPTP nucleic acid molecules, CSAPTP proteins, and anti-CSAPTP antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a

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pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for

example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of  
5 surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be  
10 brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a CSAPTP protein or anti-CSAPTP antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as  
15 required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a  
20 powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and  
25 used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the  
30 following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or

lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

5 For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be  
10 permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in  
15 the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will  
20 protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art.  
25 The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein includes physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound

5 calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

10 Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio

15 LD<sub>50</sub>/ED<sub>50</sub>. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in

20 formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially

25 from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC<sub>50</sub> (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high

30 performance liquid chromatography.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen *et al.* (1994) *PNAS* 91:3054-3057). The  
5 pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the  
10 pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

#### V. Uses and Methods of the Invention

15 The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (e.g., therapeutic and prophylactic).

20 As described herein, a CSAPTP protein of the invention has one or more of the following activities: (i) interaction of a CSAPTP protein with a CSAPTP target molecule; (ii) interaction of a CSAPTP protein with a CSAPTP target molecule, wherein the CSAPTP target is a ligand, e.g., phosphorylated amino acid residue of a phosphorylated protein, e.g., a phosphatase, for example, a cell cycle regulatory  
25 phosphatase, e.g., Cdc25A phosphatase, a cell cycle phosphatase which regulates the G1/S-phase transition, a CSAPTPase, a kinase, e.g., Mitogen Activating Protein (MAP) kinase, or a Cardiovascular Associated Protein Tyrosine Kinase (CSAPTK); (iii) interaction of a CSAPTP protein with a CSAPTP target molecule, wherein the CSAPTP target is a receptor, e.g., insulin receptor, insulin receptor substrate 1; (iv) interaction of  
30 a CSAPTP protein with a CSAPTP target molecule, wherein the CSAPTP target is a viral protein, e.g., *vaccinia* viral transcription-mediating proteins, Myxoma viral



proteins, Shope Fibroma viral proteins, *Leishmania donovani*, *Trypanosoma brucei* and *Trypanosoma cruzi* viral proteins.

Further, as described herein, a CSAPTP protein of the invention has one or more of the above activities and can thus be used in, for example, the: (1) regulation of cell cycle, e.g., dephosphorylation of phosphorylated proteins involved in the cell cycle, e.g., proliferation-mediating proteins, e.g., Cdc25A, a cell cycle phosphatase which regulates the G1/S-phase transition, e.g., anti-proliferative proteins, either *in vitro*, *in vivo* or *in situ*; (2) mediation of viral pathogenicity, e.g., viral phosphatase mediated dephosphorylation of host phosphorylated proteins, for example, viral induced disease, e.g., *Yersinia* pathogenesis, for example, *Yersinia pestis* (Bubonic Plague), e.g., viral phosphatase mediated dephosphorylation of host anti-proliferative phosphorylated proteins, for example, viral induced proliferative diseases, e.g., viral induced cancers, either *in vitro*, *in vivo* or *in situ*; (3) regulation of the phosphorylation state of receptors, e.g., insulin receptor, e.g., insulin receptor substrate 1, either *in vitro*, *in vivo* or *in situ*.

The isolated nucleic acid molecules of the invention can be used, for example, to express CSAPTP protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect CSAPTP mRNA (e.g., in a biological sample) or a genetic alteration in a CSAPTP gene, and to modulate CSAPTP activity, as described further below. The CSAPTP proteins can be used to treat disorders characterized by insufficient or excessive production of a CSAPTP or CSAPTP target molecules. In addition, the CSAPTP proteins can be used to screen for naturally occurring CSAPTP target molecules, to screen for drugs or compounds which modulate CSAPTP activity, as well as to treat disorders characterized by insufficient or excessive production of CSAPTP protein or production of CSAPTP protein forms which have decreased or aberrant activity compared to CSAPTP wild type protein. Moreover, the anti-CSAPTP antibodies of the invention can be used to detect and isolate CSAPTP proteins, regulate the bioavailability of CSAPTP proteins, and modulate CSAPTP activity.

Accordingly one embodiment of the present invention involves a method of use (e.g., a diagnostic assay, prognostic assay, or a prophylactic/therapeutic method of treatment) wherein a molecule of the present invention (e.g., a CSAPTP protein, CSAPTP nucleic acid, or a CSAPTP modulator) is used, for example, to diagnose,

prognose and/or treat a disease and/or condition in which any of the aforementioned activities (i.e., activities (i) - (iv) and (1) - (3) in the above paragraph) is indicated. In another embodiment, the present invention involves a method of use (e.g., a diagnostic assay, prognostic assay, or a prophylactic/therapeutic method of treatment) wherein a molecule of the present invention (e.g., a CSAPTP protein, CSAPTP nucleic acid, or a CSAPTP modulator) is used, for example, for the diagnosis, prognosis, and/or treatment of subjects, preferably a human subject, in which any of the aforementioned activities is pathologically perturbed. In a preferred embodiment, the methods of use (e.g., diagnostic assays, prognostic assays, or prophylactic/therapeutic methods of treatment) involve administering to a subject, preferably a human subject, a molecule of the present invention (e.g., a CSAPTP protein, CSAPTP nucleic acid, or a CSAPTP modulator) for the diagnosis, prognosis, and/or therapeutic treatment. In another embodiment, the methods of use (e.g., diagnostic assays, prognostic assays, or prophylactic/therapeutic methods of treatment) involve administering to a human subject a molecule of the present invention (e.g., a CSAPTP protein, CSAPTP nucleic acid, or a CSAPTP modulator).

A. Screening Assays:

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other drugs) which bind to CSAPTP proteins, have a stimulatory or inhibitory effect on, for example, CSAPTP expression or CSAPTP activity, or have a stimulatory or inhibitory effect on, for example, the activity of an CSAPTP target molecule.

In one embodiment, the invention provides assays for screening candidate or test compounds which are target molecules of a CSAPTP protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a CSAPTP protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological

libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994) *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a CSAPTP protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate CSAPTP activity determined. Determining the ability of the test compound to modulate CSAPTP activity can be accomplished by monitoring the bioactivity of the CSAPTP protein or biologically active portion thereof. The cell, for example, can be of mammalian origin or a yeast cell. Determining the ability of the test compound to modulate CSAPTP activity can be accomplished, for example, by coupling the CSAPTP protein or biologically active portion thereof with a radioisotope or enzymatic label such that binding of the CSAPTP protein or biologically active portion thereof to its cognate target molecule can be determined by detecting the labeled CSAPTP protein or biologically active portion thereof in a complex. For example, compounds (e.g.,

CSAPTP protein or biologically active portion thereof) can be labeled with  $^{125}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ , or  $^3\text{H}$ , either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (e.g., CSAPTP protein or biologically active portion thereof) to interact with its cognate target molecule without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with its cognate target molecule without the labeling of either the compound or the receptor. McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (e.g., Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between compound and receptor.

In a preferred embodiment, the assay comprises contacting a cell which expresses a CSAPTP protein or biologically active portion thereof, with a target molecule to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to modulate the activity of the CSAPTP protein or biologically active portion thereof, wherein determining the ability of the test compound to modulate the activity of the CSAPTP protein or biologically active portion thereof, comprises determining the ability of the test compound to modulate a biological activity of the CSAPTP expressing cell (e.g., determining the ability of the test compound to modulate cell proliferation, viral replication, and/or receptor regulation).

In another preferred embodiment, the assay comprises contacting a cell which is responsive to a CSAPTP protein or biologically active portion thereof, with a CSAPTP protein or biologically-active portion thereof, to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to modulate the activity of the CSAPTP protein or biologically active portion thereof,

wherein determining the ability of the test compound to modulate the activity of the CSAPTP protein or biologically active portion thereof comprises determining the ability of the test compound to modulate a biological activity of the CSAPTP-responsive cell (e.g., determining the ability of the test compound to modulate cell proliferation, viral replication, and/or receptor regulation).

5 In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a CSAPTP target molecule with a test compound and determining the ability of the test compound to modulate (e.g. stimulate or inhibit) the activity of the CSAPTP target molecule. Determining the ability of the test compound to modulate the activity of a CSAPTP target molecule can be accomplished, for example, by determining the ability of the CSAPTP protein to bind to or interact with the CSAPTP target molecule.

Determining the ability of the CSAPTP protein to bind to or interact with a CSAPTP target molecule can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the CSAPTP protein to bind to or interact with a CSAPTP target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting dephosphorylation of a phosphorylated protein. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (i.e. intracellular  $\text{Ca}^{2+}$ , diacylglycerol,  $\text{IP}_3$ , etc.), detecting catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, e.g., luciferase), or detecting a target-regulated cellular response, for example, cell proliferation, viral replication, and/or receptor regulation

25 In yet another embodiment, an assay of the present invention is a cell-free assay in which a CSAPTP protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the CSAPTP protein or biologically active portion thereof is determined. Binding of the test compound to the CSAPTP protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the CSAPTP protein or

biologically active portion thereof with a known compound which binds CSAPTP (e.g., a CSAPTP target molecule) to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a CSAPTP protein, wherein determining the ability of the test compound to interact with a CSAPTP protein comprises determining the ability of the test compound to preferentially bind to CSAPTP or biologically active portion thereof as compared to the known compound.

In another embodiment, the assay is a cell-free assay in which a CSAPTP protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the CSAPTP protein or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a CSAPTP protein can be accomplished, for example, by determining the ability of the CSAPTP protein to bind to a CSAPTP target molecule by one of the methods described above for determining direct binding. Determining the ability of the CSAPTP protein to bind to a CSAPTP target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (e.g., BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of a CSAPTP protein can be accomplished by determining the ability of the CSAPTP protein to further modulate the activity of a downstream effector (e.g., a transcriptionally activated immediate early response pathway component) of a CSAPTP target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting a CSAPTP protein or biologically active portion thereof with a known compound which binds the CSAPTP protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the CSAPTP protein, wherein determining the ability of the test compound to interact with the CSAPTP protein comprises determining the ability of the CSAPTP protein to preferentially bind to or modulate the activity of a CSAPTP target molecule.

The cell-free assays of the present invention are amenable to use of both soluble and/or membrane-bound forms of isolated proteins (e.g. CSAPTP proteins or biologically active portions thereof or receptors to which CSAPTP targets bind). In the case of cell-free assays in which a membrane-bound form of an isolated protein is used (e.g., a cell surface receptor) it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the isolated protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)<sub>n</sub>, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either CSAPTP or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a CSAPTP protein, or interaction of a CSAPTP protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/ CSAPTP fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre

plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or CSAPTP protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove  
5 any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of CSAPTP binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the  
10 screening assays of the invention. For example, either a CSAPTP protein or a CSAPTP target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated CSAPTP protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96  
15 well plates (Pierce Chemical). Alternatively, antibodies reactive with CSAPTP protein or target molecules but which do not interfere with binding of the CSAPTP protein to its target molecule can be derivatized to the wells of the plate, and unbound target or CSAPTP protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized  
20 complexes, include immunodetection of complexes using antibodies reactive with the CSAPTP protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the CSAPTP protein or target molecule.

In another embodiment, modulators of CSAPTP expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of  
25 CSAPTP mRNA or protein in the cell is determined. The level of expression of CSAPTP mRNA or protein in the presence of the candidate compound is compared to the level of expression of CSAPTP mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of CSAPTP expression based on this comparison. For example, when expression of CSAPTP  
30 mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a



stimulator of CSAPTP mRNA or protein expression. Alternatively, when expression of CSAPTP mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of CSAPTP mRNA or protein expression. The level of CSAPTP mRNA or protein expression in the cells can be determined by methods described herein for detecting CSAPTP mRNA or protein.

In yet another aspect of the invention, the CSAPTP proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with CSAPTP ("CSAPTP-binding proteins" or "CSAPTP-bp") and are involved in CSAPTP activity. Such CSAPTP-binding proteins are also likely to be involved in the propagation of signals by the CSAPTP proteins or CSAPTP targets as, for example, downstream elements of a CSAPTP-mediated signaling pathway. Alternatively, such CSAPTP-binding proteins are likely to be CSAPTP inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a CSAPTP protein is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a CSAPTP-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the CSAPTP protein.

This invention further pertains to novel agents identified by the above-described screening assays and to processes for producing such agents by use of these assays. Accordingly, in one embodiment, the present invention includes a compound or agent obtainable by a method comprising the steps of any one of the aforementioned screening  
5 assays (e.g., cell-based assays or cell-free assays). For example, in one embodiment, the invention includes a compound or agent obtainable by a method comprising contacting a cell which expresses a CSAPTP target molecule with a test compound and the determining the ability of the test compound to bind to, or modulate the activity of, the CSAPTP target molecule. In another embodiment, the invention includes a compound  
10 or agent obtainable by a method comprising contacting a cell which expresses a CSAPTP target molecule with a CSAPTP protein or biologically-active portion thereof, to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with, or modulate the activity of, the CSAPTP target molecule. In another embodiment, the invention includes a  
15 compound or agent obtainable by a method comprising contacting a CSAPTP protein or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to, or modulate (e.g., stimulate or inhibit) the activity of, the CSAPTP protein or biologically active portion thereof. In yet another embodiment, the present invention included a compound or agent obtainable by a method comprising  
20 contacting a CSAPTP protein or biologically active portion thereof with a known compound which binds the CSAPTP protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with, or modulate the activity of the CSAPTP protein.

Accordingly, it is within the scope of this invention to further use an agent  
25 identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a CSAPTP modulating agent, an antisense CSAPTP nucleic acid molecule, a CSAPTP-specific antibody, or a CSAPTP-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can  
30 be used in an animal model to determine the mechanism of action of such an agent.

Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

The present invention also pertains to uses of novel agents identified by the above-described screening assays for diagnoses, prognoses, and treatments as described herein. Accordingly, it is within the scope of the present invention to use such agents in the design, formulation, synthesis, manufacture, and/or production of a drug or pharmaceutical composition for use in diagnosis, prognosis, or treatment, as described herein. For example, in one embodiment, the present invention includes a method of synthesizing or producing a drug or pharmaceutical composition by reference to the structure and or properties of a compound obtainable by one of the above-described screening assays. For example, a drug or pharmaceutical composition can be synthesized based on the structure and/or properties of a compound obtained by a method in which a cell which expresses a CSAPTP target molecule is contacted with a test compound and the ability of the test compound to bind to, or modulate the activity of, the CSAPTP target molecule is determined. In another exemplary embodiment, the present invention includes a method of synthesizing or producing a drug or pharmaceutical composition based on the structure and/or properties of a compound obtainable by a method in which a CSAPTP protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to, or modulate (e.g., stimulate or inhibit) the activity of, the CSAPTP protein or biologically active portion thereof is determined.

#### B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

### 1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the CSAPTP  
5 nucleotide sequences, described herein, can be used to map the location of the CSAPTP genes on a chromosome. The mapping of the CSAPTP sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, CSAPTP genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the CSAPTP nucleotide sequences.

10 Computer analysis of the CSAPTP sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the CSAPTP sequences will yield an amplified fragment.

15 Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains  
20 the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. *et al.* (1983) *Science* 220:919-924). Somatic cell  
25 hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the CSAPTP nucleotide  
30 sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can

similarly be used to map a 90, 1p, or 1v sequence to its chromosome include *in situ* hybridization (described in Fan, Y. *et al.* (1990) *PNAS*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

5           Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A  
10       pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will  
15       suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for  
20       marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the  
25       physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes),  
30       described in, for example, Egeland, J. *et al.* (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the CSAPTP gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease.

- 5 Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

10

## 2. Tissue Typing

- The CSAPTP sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for
- 15 identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers
- 20 for RFLP (described in U.S. Patent 5,272,057).

- Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the CSAPTP nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the
- 25 sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

- Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the
- 30 present invention can be used to obtain such identification sequences from individuals and from tissue. The CSAPTP nucleotide sequences of the invention uniquely represent

portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:12 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:12 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from CSAPTP nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

20

### 3. Use of Partial CSAPTP Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology.

Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:12 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the CSAPTP nucleotide sequences or portions thereof, e.g., fragments derived from the noncoding regions of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:12 having a length of at least 20 bases, preferably at least 30 bases.

The CSAPTP nucleotide sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, e.g., brain tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such CSAPTP probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, e.g., CSAPTP primers or probes can be used to screen tissue culture for contamination (i.e. screen for the presence of a mixture of different types of cells in a culture).

#### C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining CSAPTP protein and/or nucleic acid expression as well as CSAPTP



activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant CSAPTP expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with CSAPTP protein, nucleic acid expression or activity. For example, mutations in a CSAPTP gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with CSAPTP protein, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of CSAPTP in clinical trials.

These and other agents are described in further detail in the following sections.

#### 1. Diagnostic Assays

An exemplary method for detecting the presence or absence of CSAPTP protein or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting CSAPTP protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes CSAPTP protein such that the presence of CSAPTP protein or nucleic acid is detected in the biological sample. A preferred agent for detecting CSAPTP mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to CSAPTP mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length CSAPTP nucleic acid, such as the nucleic acid of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 or SEQ ID NO:10 (or that of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12, or the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, or \_\_\_\_\_, or a portion thereof), such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to CSAPTP mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting CSAPTP protein is an antibody capable of binding to CSAPTP protein, preferably an antibody with a detectable label. Antibodies

can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect CSAPTP mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of CSAPTP mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of CSAPTP protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of CSAPTP genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of CSAPTP protein include introducing into a subject a labeled anti-CSAPTP antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting CSAPTP protein, mRNA, or genomic DNA, such that the presence of CSAPTP protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of CSAPTP protein, mRNA or genomic DNA in the control sample with the presence of CSAPTP protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of CSAPTP in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting CSAPTP protein or mRNA in a biological sample; means for determining the amount of CSAPTP in the sample; and means for comparing the amount of CSAPTP in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect CSAPTP protein or nucleic acid.

## 2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant CSAPTP expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with CSAPTP protein, nucleic acid expression or activity such a proliferative disorder (e.g., cancer). Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a metabolic disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant CSAPTP expression or activity in which a test sample is obtained from a subject and CSAPTP protein or nucleic acid (e.g., mRNA, genomic DNA) is detected, wherein the presence of CSAPTP protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant CSAPTP expression or activity. As used herein, a "test sample" includes a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant CSAPTP expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a proliferative disorder (e.g., cancer). For example, such methods can be used to determine whether a subject can be effectively treated with

an agent for a metabolic disorder. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant CSAPTP expression or activity in which a test sample is obtained and CSAPTP protein or nucleic acid expression or activity is detected (e.g.,  
5 wherein the abundance of CSAPTP protein or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant CSAPTP expression or activity.)

The methods of the invention can also be used to detect genetic alterations in a CSAPTP gene, thereby determining if a subject with the altered gene is at risk for a  
10 disorder characterized by aberrant developmental progression. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a CSAPTP-protein, or the mis-expression of the CSAPTP gene. For example, such genetic alterations can be detected by  
15 ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a CSAPTP gene; 2) an addition of one or more nucleotides to a CSAPTP gene; 3) a substitution of one or more nucleotides of a CSAPTP gene, 4) a chromosomal rearrangement of a CSAPTP gene; 5) an alteration in the level of a messenger RNA transcript of a CSAPTP gene, 6) aberrant modification of a CSAPTP gene, such as of  
20 the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a CSAPTP gene, 8) a non-wild type level of a CSAPTP-protein, 9) allelic loss of a CSAPTP gene, and 10) inappropriate post-translational modification of a CSAPTP-protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting alterations  
25 in a CSAPTP gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a  
30 ligation chain reaction (LCR) (see, e.g., Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *PNAS* 91:360-364), the latter of which can be particularly

useful for detecting point mutations in the CSAPTP-gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more  
5 primers which specifically hybridize to a CSAPTP gene under conditions such that hybridization and amplification of the CSAPTP-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in  
10 conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, 1990, *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.*, 1989, *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.*, 1988, *Bio/Technology* 6:1197), or any  
15 other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a CSAPTP gene from a sample cell  
20 can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence  
25 specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in CSAPTP can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, to high density  
30 arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-

759). For example, genetic mutations in CSAPTP can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the CSAPTP gene and detect mutations by comparing the sequence of the sample CSAPTP with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert ((1977) *PNAS* 74:560) or Sanger ((1977) *PNAS* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, e.g., PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the CSAPTP gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type CSAPTP sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with

hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in CSAPTP cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from *Hel. a* cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a CSAPTP sequence, e.g., a wild-type CSAPTP sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in CSAPTP genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat Res* 285:125-144; and Hayashi (1992) *Genet Anal Tech Appl* 9:73-79). Single-stranded DNA fragments of sample and control CSAPTP nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex

molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing  
5 gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control  
10 and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions  
15 which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

20 Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme  
25 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for  
30 amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it



possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose  
5 patients exhibiting symptoms or family history of a disease or illness involving a CSAPTP gene.

Furthermore, any cell type or tissue in which CSAPTP is expressed may be utilized in the prognostic assays described herein.

10

### 3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of a CSAPTP protein (*e.g.*, modulation of cell proliferation, *e.g.*, dephosphorylation of phosphorylated cell cycle proteins) can be applied not only in  
15 basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase CSAPTP gene expression, protein levels, or upregulate CSAPTP activity, can be monitored in clinical trials of subjects exhibiting decreased CSAPTP gene expression, protein levels, or downregulated CSAPTP activity. Alternatively, the effectiveness of an agent  
20 determined by a screening assay to decrease CSAPTP gene expression, protein levels, or downregulate CSAPTP activity, can be monitored in clinical trials of subjects exhibiting increased CSAPTP gene expression, protein levels, or upregulated CSAPTP activity. In such clinical trials, the expression or activity of a CSAPTP gene, and preferably, other genes that have been implicated in, for example, a developmental disorder can be used  
25 as a "read out" or markers of the phenotype of a particular cell.

For example, and not by way of limitation, genes, including CSAPTP, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates CSAPTP activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on proliferative disorders,  
30 for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of CSAPTP and other genes implicated in a proliferative,

differentiative, or metabolic disorder. The levels of gene expression (i.e., a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of CSAPTP or other  
5 genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for  
10 monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a CSAPTP protein, mRNA, or genomic  
15 DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the CSAPTP protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the CSAPTP protein, mRNA, or genomic DNA in the pre-administration sample with the CSAPTP protein, mRNA, or  
20 genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of CSAPTP to higher levels than detected, i.e., to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease  
25 expression or activity of CSAPTP to lower levels than detected, i.e. to decrease the effectiveness of the agent. According to such an embodiment, CSAPTP expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

### C. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant CSAPTP expression or activity. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, includes the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the CSAPTP molecules of the present invention or CSAPTP modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

#### 1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant CSAPTP expression or activity, by administering to the subject a CSAPTP or an agent which modulates CSAPTP expression or at least one CSAPTP activity. Subjects at risk for a disease which is caused or contributed to by aberrant CSAPTP expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the CSAPTP aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of CSAPTP aberrancy, for example, a CSAPTP, CSAPTP agonist or CSAPTP antagonist agent can be used for treating the subject. The appropriate agent can be determined

based on screening assays described herein. The prophylactic methods of the present invention are further discussed in the following subsections.

## 2. Therapeutic Methods

5 Another aspect of the invention pertains to methods of modulating CSAPTP expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a CSAPTP or agent that modulates one or more of the activities of CSAPTP protein activity associated with the cell. An agent that modulates CSAPTP protein activity can  
10 be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a CSAPTP protein, a CSAPTP antibody, a CSAPTP agonist or antagonist, a peptidomimetic of a CSAPTP agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more CSAPTP activities. Examples of such stimulatory agents include active CSAPTP protein and a nucleic acid  
15 molecule encoding CSAPTP that has been introduced into the cell. In another embodiment, the agent inhibits one or more CSAPTP activities. Examples of such inhibitory agents include antisense CSAPTP nucleic acid molecules, anti-CSAPTP antibodies, and CSAPTP inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent), *in vivo* (e.g., by administering the agent  
20 to a subject), or alternatively *in situ* (e.g., at the site of lesion or injury, for example, in the hematopoietic system, e.g., bone marrow). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a CSAPTP protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a  
25 screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) CSAPTP expression or activity. In another embodiment, the method involves administering a CSAPTP protein or nucleic acid molecule as therapy to compensate for reduced or aberrant CSAPTP expression or activity.

Stimulation of CSAPTP activity is desirable in situations in which CSAPTP is  
30 abnormally downregulated and/or in which increased CSAPTP activity is likely to have a beneficial effect. For example, stimulation of CSAPTP activity is desirable in

situations in which a CSAPTP is downregulated and/or in which increased CSAPTP activity is likely to have a beneficial effect. Likewise, inhibition of CSAPTP activity is desirable in situations in which CSAPTP is abnormally upregulated and/or in which decreased CSAPTP activity is likely to have a beneficial effect.

5

### 3. Pharmacogenomics

The CSAPTP molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on CSAPTP activity (e.g., CSAPTP gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders associated with aberrant CSAPTP activity (e.g., proliferative and/or differentiative disorder). In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a CSAPTP molecule or CSAPTP modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a CSAPTP molecule or CSAPTP modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See e.g., Eichelbaum, M., *Clin Exp Pharmacol Physiol*, 1996, 23(10-11):983-985 and Linder, M.W., *Clin Chem*, 1997, 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the

main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution  
5 map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers  
10 associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may  
15 be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among  
20 such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drugs target is known (e.g., a CSAPTP protein or CSAPTP receptor of the present invention), all common variants of that gene can be fairly easily identified in the  
25 population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT  
30 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated

drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., a CSAPTP molecule or CSAPTP modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a CSAPTP molecule or CSAPTP modulator, such as a modulator identified by one of the exemplary screening assays described herein.

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This invention is further illustrated by the following examples which should not be construed as limiting. The contents of Sequence Listing, figures and all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

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## EXAMPLES

**EXAMPLE 1: IDENTIFICATION AND CHARACTERIZATION OF  
HUMAN CSAPTP-1, CSAPTP-2, and CSAPTP-3 cDNAs**

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In this example, the identification and characterization of the genes encoding human CSAPTP-1, CSAPTP-2, CSAPTP-3 and CSAPTP-4 (also referred to as b003g03, b037d02, b067c02 and fchrb018a01, respectively) is described.

10 Isolation of the human CSAPTP cDNA

The invention is based, at least in part, on the discovery of four human genes encoding members of the CSAPTP family. The human CSAPTP family members were isolated from cDNA libraries which were prepared from tissue obtained from subjects suffering from congestive heart failure of ischemic and idiopathic origin. Briefly, a  
15 cardiac tissue sample was obtained from a biopsy of a patient suffering from congestive heart failure. mRNA was isolated from the cardiac tissue and a cDNA library was prepared therefrom using art known methods (described in, for example, *Molecular Cloning: A Laboratory Manual*, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989). Positive clones were isolated either by  
20 examining the top protein blast hit for each sequence, by blasting the libraries against known phosphatases, or by using a computer program that recognizes protein motifs of phosphatases.

The sequences of the positive clones were determined and found to contain open reading frames. The nucleotide sequences encoding the human CSAPTP-1, CSAPTP-2,  
25 CSAPTP-3 and CSAPTP-4 proteins, respectively, are shown in Figures 1, 2, 3 and 15, and are set forth as SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, and SEQ ID NO:10. The full length proteins encoded by these nucleic acids comprise about 173, 263, 209 and 221 amino acids respectively, and have the amino acid sequences shown in Figures 1, 2, 3 and 15, respectively, and set forth as SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8  
30 and SEQ ID NO:11, respectively. The coding region (open reading frame) of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:10 is set forth as SEQ ID NO:3,



SEQ ID NO:6, SEQ ID NO:9 and SEQ ID NO:12, respectively. The clones comprising the entire coding region of human CSAPTP-1, CSAPTP-2, CSAPTP-3 and CSAPTP-4 were deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_ 1998, and assigned Accession Nos. \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_ respectively.

#### Analysis of human CSAPTP

A BLAST search (Altschul et al. (1990) *J. Mol. Biol.* 215:403) of the nucleotide and protein sequences of human CSAPTP-1 revealed that CSAPTP-1 is similar to the following protein: human protein tyrosine phosphatase (Accession No. AAB40597). The CSAPTP-1 nucleic acid sequence is approximately 38.7% identical to that of human protein tyrosine phosphatase, as is shown in Figure 4. The CSAPTP-1 protein sequence is approximately 78.6 % identical (over amino acids 1-173) to that of human protein tyrosine phosphatase, as is shown in Figure 5.

CSAPTP-2 is similar to the following protein: dual specificity protein phosphatase VHR (Accession No. P51452). The CSAPTP-2 protein is approximately 22.5% identical (over amino acids 1-263) to dual specificity protein phosphatase VHR, as is shown in Figure 6.

CSAPTP-3 is similar to the following proteins: dual specificity phosphatase 2 (Accession No. B57126, Q05922), *C. Elegans* protein F28C6.8 (Accession No. Z68315) and *Ovis aries* protein BIIIA3 (Accession No. U60024). The CSAPTP-3 protein is approximately 50 % identical (over amino acids 118-155) and 29% identical (over amino acids 54-97) to mouse dual specificity phosphatase 2. The CSAPTP-3 protein is approximately 46 % identical (over amino acids 6-20 and 50-64) and 41% identical (over amino acids 20-36) to BIIIA3. The CSAPTP-3 protein is approximately 50 % identical (over amino acids 183-194), 42% identical (over amino acids 92-155) and 39% identical (over amino acids 60-92) to F28C6.8, as is shown in Figure 7.

CSAPTP-4 is similar to the following protein: dual specificity protein phosphatase 3 (Accession No. P51452). The CSAPTP-4 protein is approximately 42% identical (over amino acids 1-221 of SEQ ID NO: 11) to dual specificity protein phosphatase VHR, as shown in Figure 10.

5

#### Tissue Distribution of CSAPTP mRNA

This example describes the tissue distribution of CSAPTP mRNA, as determined by Northern blot hybridizations.

Northern blot hybridizations with the various RNA samples were performed under standard conditions and washed under stringent conditions, i.e., 0.2 X SSC at 65° C. DNA probes corresponding to CSAPTP-1 (Achr003g03), CSAPTP-2 (fchr037d02), CSAPTP-3 (fchr067c02) and CSAPTP-4 (fchr018a01) were used. The DNA was radioactively labeled with <sup>32</sup>P-dCTP using the Prime-It kit (Stratagene, La Jolla, CA) according to the instructions of the supplier. Filters containing human mRNA (MultiTissue Northern I and MultiTissue Northern II from Clontech, Palo Alto, CA) were probed in ExpressHyb hybridization solution (Clontech) and washed at high stringency according to manufacturer's recommendations.

The expression of CSAPTP-1 in various human and rat tissues was assessed; CSAPTP-1 message was detected in human heart and skeletal muscle, where it was highly expressed. CSAPTP-1 mRNA was also found in all rat tissues tested, but was predominantly expressed in rat skeletal muscle, heart, placenta, lung and brain.

CSAPTP-2 message was detected in several human tissues, with highest expression in skeletal muscle and moderate expression in heart and brain. In rat tissues, expression of CSAPTP-2 mRNA was mainly found in skeletal muscle.

CSAPTP-3 message was detected in several human tissues, with highest expression in heart and skeletal muscle, and moderate expression in brain, placenta, kidney and pancreas.

CSAPTP-4 message was detected in human heart and skeletal muscle. CSAPTP-4 mRNA was also expressed in several rat tissues, although most predominantly in rat brain.

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## EXAMPLE 2:       EXPRESSION OF RECOMBINANT CSAPTP-1 PROTEIN IN C2C12 CELLS

### Preparation of Transgenic C2C12 Cells

5       To express the CSAPTP-1 gene in C2C12 (mouse myoblast) cells, the CSAPTP-1 cDNA was amplified by PCR and cloned into the pcDNA/Amp vector (Invitrogen Corporation (San Diego, CA)). This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. C2C12 cells were  
10 subsequently transfected with the CSAPTP-1-pcDNA/Amp plasmid DNA. Suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

### 15       Expression of Recombinant CSAPTP-1 Protein in Transgenic C2C12 Cells

      The expression of the CSAPTP-1 polypeptide in transfected cells is detected by radiolabeling ( $^{35}\text{S}$ -methionine or  $^{35}\text{S}$ -cysteine, available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988).  
20 Briefly, the cells are labeled for 8 hours with  $^{35}\text{S}$ -methionine (or  $^{35}\text{S}$ -cysteine). The culture medium is then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). The CSAPTP-1 polypeptide is immunoprecipitated from the cell lysate and the culture medium and analyzed by SDS-PAGE.

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### Phenotype of C2C12 Cells Expressing CSAPTP-1

      C2C12 cells are myoblasts, and, upon confluency, are able to rapidly differentiate to form contractile myotubes. This myotube formation is known to be stimulated by the utilization of horse serum (as opposed to fetal bovine serum) in the  
30 medium. To determine whether the expression of the CSAPTP-1 gene in C2C12 cells impacts the ability of the cells to differentiate into myotubes, both untransfected control

C2C12 cells and a stably transfected CSAPTP-1-containing C2C12 cell line were grown for 5 days in standard growth medium containing 20% fetal bovine serum. After 5 days the medium was replaced with medium containing 2% horse serum, and the ability of the cells to fuse into myotubes was assessed. The untransfected control cells elongated and fused, forming myotubes. In contrast, the CSAPTP-1 containing cells displayed a reduced ability to fuse and formed many fewer higher-order myotube structures.

### EXAMPLE 3: GENERATION OF CSAPTP-1 TRANSGENIC ANIMALS

An N-terminal His-tagged CSAPTP-1 cDNA was ligated to the cardiac-specific alpha-myosin heavy chain promoter and the human growth hormone polyadenylation signal sequence using art known techniques. This construct was microinjected into the male pronucleus of fertilized mouse eggs, which were subsequently implanted into a pseudopregnant foster mother. Founder mice were bred with non-transgenic cohorts for the propagation of individual lines.

The presence of the CSAPTP-1 transgene in transgenic mice was confirmed by Southern analysis of genomic DNA. Briefly, 10 micrograms of mouse genomic DNA was digested with EcoRI or BamHI or XbaI (New England Biolabs), electrophoresed in a 0.8% agarose gel and transferred to a nylon membrane (Genescreen, DuPont). Membranes were hybridized with a <sup>32</sup>P-labeled cDNA probe specific for the CSAPTP-1 sequence. Results confirmed that both of the founder mice 03645-4.7F and 03650-3.8F were found to have significant levels of the CSAPTP-1 transgene, whereas transgene expression was not visible in the wild-type mouse genomic DNA.

The expression of the CSAPTP-1 gene in the transgenic mice was also assessed by Northern analysis. Total RNA from both wild-type and transgenic mice was isolated using the guanidinium isothiocyanate/acid phenol procedure (Chomczynski, (1987) Anal. Biochem. 162: 156-159). Northern blots (Sambrook (1989) *ibid*) were performed with 20 micrograms of total RNA from the heart tissue fractionated in a 1.5% agarose/formaldehyde gel and blotted onto a nylon membrane (Genescreen, DuPont). Hybridization and washing were performed as recommended by the supplier. Briefly, membranes were probed using a <sup>32</sup>P-labeled probe for CSAPTP-1, applied in 50%

formamide hybridization solution at 42°C for 18h. The results demonstrated that there is a significant amount of mRNA for the transgene in both the 03645-4.7F and 03650-3.8F founder mice, whereas message was undetectable in the wild type mouse.

The phenotypes of the transgenic mice were also assessed. The transgenic animals showed a 37% increase in heart/body ratio as compared to wild type mice, indicating that the heart was enlarged. Similarly, a significant number of the cardiomyocytes in transgenic mouse hearts were hypertrophied. An examination of the heart tissue from transgenic mice demonstrated the presence of calcification, similar to that seen in advanced stages of cardiomyopathy.

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**EXAMPLE 4:        ASSESSMENT OF THE EFFECT OF CSAPTP-1  
                      EXPRESSION ON GENE EXPRESSION**

To determine whether the expression of CSAPTP-1 had an effect on the expression of certain other genes of interest, (e.g., oncogenes, tumor suppressors, cell cycle regulators, transcription factors, cell surface antigens, or intracellular signal transduction modulators and effectors, or those genes involved in stress response, ion channels and transport, apoptosis, DNA repair, receptors, or cell-cell communications), a cDNA expression array was performed using an cDNA expression array kit (Clontech) according to the manufacturer instructions. In this procedure, labeled cDNA probe mixtures were synthesized by reverse transcription from the RNA population of whole heart tissue from both a wild type mouse and from mice containing the CSAPTP-1 transgene. Each labeled probe mixture was hybridized to an array of cDNAs for the gene of interest, the arrays were subjected to a high-stringency wash, and the hybridized labeled probes were detected and quantified by phosphorimaging. The gene levels were normalized to GADPH expression.

The expression of a number of genes was different between the wild-type and the CSAPTP-1 transgenic mice. The results of an additional cDNA expression array experiment utilizing probes synthesized from control C2C12 cells and C2C12 cells containing the CSAPTP-1 transgene also indicated that a number of genes were affected by the expression of CSAPTP-1 (see Figure 8). A 7.5-fold decrease in the presence of

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the transcript for the DB-1 gene was observed in C2C12 cells expressing the CSAPTP-1 gene as compared to control cells. DB-1 is a zinc finger transcription factor which was first cloned by its affinity to an element *cis* to the IL-3 promoter (nucleotides -76 to -47), a region which is rich in CT/GC. This region is known to bind Egr1 and Egr2, which, in the presence of TPA, transactivates the IL-3 promoter. The association of DB-1 and Egr1 or Egr2 has not been demonstrated to transactivate the IL-3 promoter. DB-1 is ubiquitously expressed, and has been shown to interact with both Tax and with prenylated RhoB. Upon binding to Tax, DB-1 is able to synergistically activate the IL-3 promoter. The interaction of DB-1 with prenylated RhoB has been implicated in actin regulation, cell growth (e.g., transformation), gene activation (e.g., SREs), and cell adhesion and motility (e.g., integrins).

In addition, a decrease in transcription of the L1CAM and CD27 genes was detected in the CSAPTP-1 transgenic C2C12 cells in comparison with wild-type cells. In contrast, the transcription of the PLA2, caspase, thymosin, and Hox7 genes was increased in the CSAPTP-1 transgenic C2C12 cells as compared to control C2C12 cells.

#### EXAMPLE 5: EXPRESSION OF RECOMBINANT CSAPTP PROTEIN IN BACTERIAL CELLS

In this example, CSAPTP is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, CSAPTP is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-CSAPTP fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

## Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following

5 claims.

What is claimed:

1. An isolated nucleic acid molecule selected from the group consisting of:
  - a) a nucleic acid molecule comprising a nucleotide sequence which  
5 is at least 38.7%, 50%, 50% or 50% homologous to a nucleotide sequence of  
SEQ ID NO:1 or 3, SEQ ID NO:4 or 6, SEQ ID NO:7 or 9, or SEQ ID NO:10 or  
12, respectively, the DNA insert of the plasmid deposited with ATCC as  
Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, or a complement thereof;
  - b) a nucleic acid molecule comprising a fragment of at least 994,  
10 626, 531 or 241 nucleotides of a nucleic acid comprising the nucleotide sequence  
of SEQ ID NO:1 or 3, SEQ ID NO:4 or 6, SEQ ID NO:7 or 9, or SEQ ID NO:10  
or 12, respectively, the DNA insert of the plasmid deposited with ATCC as  
Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, or a complement thereof;
  - c) a nucleic acid molecule which encodes a polypeptide comprising  
15 an amino acid sequence at least 79%, 23%, 24% or 42% homologous to the  
amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID  
NO:11, respectively, or an amino acid sequence encoded by the DNA insert of  
the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_,  
or \_\_\_\_\_;
  - d) a nucleic acid molecule which encodes a fragment of a  
20 polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID  
NO:5, SEQ ID NO:8 or SEQ ID NO:11, or the polypeptide encoded by the DNA  
insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_,  
\_\_\_\_\_, or \_\_\_\_\_, wherein the fragment comprises at least 15 contiguous amino  
25 acid residues of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ  
ID NO:8 or SEQ ID NO:11, or the polypeptide encoded by the DNA insert of the  
plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or  
\_\_\_\_\_; and
  - e) a nucleic acid molecule which encodes a naturally occurring  
30 allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID  
NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or an amino acid



sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:12 under stringent conditions.

2. The isolated nucleic acid molecule of claim 1 which is selected from the group consisting of:

a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12 or the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, or a complement thereof; and

b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_.

3. The nucleic acid molecule of claim 1 further comprising vector nucleic acid sequences.

4. The nucleic acid molecule of claim 1 further comprising nucleic acid sequences encoding a heterologous polypeptide.

5. A host cell which contains the nucleic acid molecule of claim 1.

6. The host cell of claim 5 which is a mammalian host cell.

7. A non-human mammalian host cell containing the nucleic acid molecule of claim 1.

8. An isolated polypeptide selected from the group consisting of:

- 5 a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or the polypeptide encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_;
- 10 b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:12 under stringent conditions; and
- 15 c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 38.7%, 50%, 50% or 50% homologous to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1 or 3, SEQ ID NO:4 or 6, SEQ ID NO:7 or 9, or SEQ ID NO:10 or 12, respectively, the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_.
- 20 d) a polypeptide comprising an amino acid sequence which is at least 79%, 23%, 24% or 42% homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, respectively, or the polypeptide encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_.
- 25
- 30

9. The isolated polypeptide of claim 8 comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_.

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10. The polypeptide of claim 8 further comprising heterologous amino acid sequences.

11. An antibody which selectively binds to a polypeptide of claim 8.

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12. A method for producing a polypeptide selected from the group consisting of:

a) a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_;

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b) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_ wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_; and

20

c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID

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NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:12 under stringent conditions;

comprising culturing the host cell of claim 5 under conditions in which the nucleic acid molecule is expressed.

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13. A method for detecting the presence of a polypeptide of claim 8 in a sample comprising:

- a) contacting the sample with a compound which selectively binds to the polypeptide; and
- 10 b) determining whether the compound binds to the polypeptide in the sample to thereby detect the presence of a polypeptide of claim 8 in the sample.

14. The method of claim 13, wherein the compound which binds to the  
15 polypeptide is an antibody.

15. A kit comprising a compound which selectively binds to a polypeptide of claim 8 and instructions for use.

20 16. A method for detecting the presence of a nucleic acid molecule in claim 1 in a sample comprising:

- a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and
- 25 b) determining whether the nucleic acid probe or primer binds to a nucleic acid molecule in the sample to thereby detect the presence of a nucleic acid molecule of claim 1 in the sample.

17. The method of claim 16, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

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18. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of claim 1 and instructions for use.

19. A method for identifying a compound which binds to a polypeptide of claim 8 comprising:

- a) contacting the polypeptide, or a cell expressing the polypeptide with a test compound; and
- b) determining whether the polypeptide binds to the test compound.

20. The method of claim 19, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

- a) detection of binding by direct detection of test compound/polypeptide binding;
- b) detection of binding using a competition binding assay; and
- c) detection of binding using an assay for CSAPTP activity.

21. A method of modulating the activity of a polypeptide of claim 8 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

22. A method for identifying a compound which modulates the activity of a polypeptide of claim 8 comprising:

- a) contacting a polypeptide of claim 8 with a test compound; and
- b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

1/14

**DNA sequence**

GTCGACCCACGCGTCCGTGGGTTTTCTTTTTTAATTATCCAAACAGTGGGCAGCTTC  
CTCCCCCACACCCAAGTATTTGCACAATATTTGTGCGGGGTATGGGGGTGGGTTTTT  
AAATCTCGTTTTCTCTTGGACAAGCACAGGGATCTCGTTCTCCTCATTTTTTGGGGGTG  
TGTGGGGACTTCTCAGGTCGTGTCCCCAGCCTTCTCTGCAGTCCCTTCTGCCCTGC  
CGGGCCCCGTGCGGAGGCGCCATGGCTCGGATGAACCGCCCGGCCCGGGTGGAG  
GTGAGCTACAAACACATGCGCTTCTCATCACCCACAACCCACCAACGCCACGCT  
CAGCACCTTCATTGAGGACCTGAAGAAGTACGGGGCTACCACTGTGGTGCGTGTGT  
GTGAAGTGACCTATGACAAAACGCCGCTGGAGAAGGATGGCATCACCGTTGTGGAC  
TGGCCGTTTGACGATGGGGCGCCCCCGCCGCAAGGTAGTGAAGACTGGCTG  
AGCCTGGTGAAGGCCAAGTTCTGTGAGGCCCCCGGCAGCTGCGTGGCTGTGCACT  
GCGTGGCGGGCCTGGGCGGGCTCCAGTCCTTGTGGCGCTGGCCCTTATTGAGA  
GCGGGATGAAGTACGAGGACGCCATCCAGTTCATCCGCCAGAAGCGCCGCGGAG  
CCATCAACAGCAAGCAGCTCACCTACCTGGAGAAATACCGGCCAAACAGAGGCTG  
CGGTTCAAAGACCCACACACGCACAAGACCCGGTGCTGCGTTATGTAGCTCAGGAC  
CTTGGCTGGGCCTGGTCGTCATGTAGGTCAGGACCTTGGCTGGACCTGGAGGCCC  
TGCCAGCCCTGCTCTGCCCAGCCCAGCAGGGGCTCCAGGCCTTGGCTGGCCCC  
ACATCGCCTTTTCTCCCCGACACCTCCGTGCACTTGTGTCCGAGGAGCGAGGAGC  
CCCTCGGGCCCTGGGTGGCCTCTGGGCCCTTTCTCCTGTCTCCGCCACTCCCTCT  
GGCGGCGCTGGCCGTGGCTCTGTCTCTCTGAGGTGGGTGCGGGCGCCCTCTGCCC  
GCCCCCTCCACACCAGCCAGGCTGGTCTCCTCTAGCCTGTTTGTGTGGGGTGG  
GGGTATATTTTGTAACTGAGGCCCCAGCCCTCTTTTGCAGCCCTTGTCTCTGA  
CCTGTTCTCGGCACCTTAAATTATTAGACCCCGGGGCAGTCAGGTGCTCCGGACAC  
CCGAAGGCAATAAAACAGGAGCCGTGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA  
AAAAAAAAAAAAAAAAAAAAAAAAAAGGGCGGCCGN

**amino acid sequence**

MARMNRPAPVEVSYPKHMRLITHNPTNATLSTFIEDLKKGATTVVRVCEVTYDKTPLE  
KDGITVVDWPFDDGAPPGKVVEDWLSLVKAKFCEAPGSCVAVHCVAGLGRAPVLVA  
LALIESGMKYEDAIQFIRQKRRGAINSKQLTYLEKYRPKQRLRFKDPHTHKTRCCVM

**FIGURE 1**

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**DNA sequence**

GTGACCCACGCGTCCGGCGGCTCCTCTACACAGGCAAGACAGCCTGTAACCATGCCGAC  
GAGGTCTGGCCAGGCCTCTATCTCGGAGACCAGGACATGGCTAACAACCGCCGGGAGCTT  
CGCCGCCTGGGCATCACGCACGTCTCAATGCCTCACACAGCCGGTGGCGAGGCACGCCC  
GAGGCCTATGAGGGGCTGGGCATCCGCTACCTGGGTGTGAGGCCCACGACTCGCCAGCC  
TTTGACATGAGCATCCACTTCCAGACGGCTGCCGACTTCATCCACCGGGCGCTGAGCCAG  
CCAGGAGGGAAGATCCTGGTGCAATTGTGCTGTGGGCGTGAGCCGATCCGCCACCTTGGTA  
CTGGCCTACCTCATGCTGTACCACCACCTTACCCTCGTGGAGGCCATCAAGAAAGTCAAA  
GACCACCGAGGAGAGGCCGAGCCCCAGGCCACTGTCACTCTTTGTGGGAGGGGACGGGGA  
GTGAGGTTGGGCAGTGTGGTGGATGGGCACCCAGGAAGGGTTGACCAGGGAAGGAGGCAG  
CTAGGCTGTAGATGGAAGATGGTCCCTGGGATTTCGAACACCGCTGGGATCTGGCCAGGGTG  
CTCCCTGGGATTCACAGTCCCTTCCCCCTCTTGTGCCCCAAGTGTTCCTCTCTCCCTCA  
CCAAAACAAAAAGGGCCATCTCTGCCCCTGCACTTTGTGCAGAAAGTCAGGGATACGGC  
AAGCATGAATGCAATGGTGTAGAGTTGTGTGAAACCCCTAGCATAGAGACAGACAGCGAA  
GAGATGGTGTGAAAAGCTTGCAGAACACAGACAGAGAACCCACAGACTTTCCACTCCAAG  
CACAGGAGGAGGTAGCTAGCGTGTGAGGGTTGGCACTAGGCCACGGCTGCTGCTTGGGC  
CAAAAACATACAGAGGTGCATGGCTGGCAGTCTTGAATTGTCACTCGCTTACTGGATCC  
AAGCGTCTCGAGGATAAATAAAGATCATGAAAAAAAAAAAAAAAAAAGGGCGGCCGC

**amino acid sequence**

VDPRVRRLLYTGKTACNHADEVWPGLYLGDQDMANNRRELRLRGITHVLNASHSRWRGTP  
EAYEGLGIRYLGVEAHDSPAFDMSIHFQTAADFIHRALSQPGGKILVHCAVGVSRSATLV  
LAYLMLYHHLTLVEAIKKVKDHRGEAEPQATVTLGRGRGVRLGSVVDGHPGRVDQGRRO  
LGCRWKMLVGFHRWDLARVLPGIHSPFSLCPSVSLSPSPKTKRAISAPALCAESQGYG  
KHECNGVELCETPSIETDSEEMV

**FIGURE 2**

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## dna sequence

TN GGATCGAMCNSGCGTCCGGGCGGGCCCCGCGCTGCTGGAGGCCGGCCTGGCGCGGGTGCTCTTCTACCGACGCTGCTCT  
ACACCCTGTTCCGCGGGAAGGTGCCGGGTCCGGGCGACCGGGACTGGTACCACCGCATCGACCCACCGTGCTGCTGGGCG  
CGCTGCCGTTGCGGAAGCTTGACGCGCCAGCTGGTACAGGACGAGAACGTGCGCGGGTGATCACCATGAACGAGGAGTAC  
GAGACGAGGTTCTCTGTGCAACTCTTACAGGAGTGGAAGAGACTAGGAGTCGAGCAGCTGCGGCTCAGCACAGTAGACATG  
ACTGGGATCCCCACCTTGGACAACCTCCAGAAGGGAGTCCAATTGCTCTCAAGTACCACTCGCTGGGCCAGTGTGTTTAC  
GTGCATGTGAAGGCTGGGCGCTCCAGGAGTGCCACTATGGTGGCAGCATACCTGATTCAGGTGTCACAAATGGGAGTCCAG  
AGGAGGCTGTAAGAGCCATCGCCCAAGATCCGGGTCATACATCCACATCAGGCCTGGCCAGCTTGGATGTTCTTAAAGAGT  
TCCACAAAGCAGATTACTGCACSGGCMACAAAGGATGGGACTTTTGKCATTTCAAAGACATGATGTATGGGGATTAGAAAG  
AACTCAAGACACTCCTGCTTGATACAGAACAAAAGAGCTTAACAGGACCAACAGGGCTTAAGCCCAGACTTGACGTAACA  
GAAATGTGCCAATAGGTAATAGGTAATTTTCTTCTGACTTGTTTGTCTTCTNAAATGGCACTGTTGAANNANTNTN  
NCTC

## protein sequence

XDRXXVRAAPRCWRPAWRGCSSTRRCSTPCSAGRCRVGRGTGTASTPPCCWARCRCSLTRLVQDENVRGVITMNEE  
YETRFLCNSSQEWKRLGVEQLRLSTVDMTGIPDLNLQKGVQFALKYQSLGQCQVYVHCKAGRSRSATMVAAYLIQVAQMG  
VQRRLEPSPKIRVIHPHQAWPAWMFLKSSTKQITAXATKDGTFXISK

*FIGURE 3*



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ALIGN calculates a global alignment of two sequences  
 version 2.0u Please cite: Myers and Miller, CABIOS (1989)  
 > Achrb003g03cons - Vector Check 1315 aa vs.  
 > Genbank U48296 - Human protein tyrosine phosphatase 2200 aa  
 scoring matrix: paml20.mat, gap penalties: -12/-4  
 38.7% identity; Global alignment score: 49

```

              10      20      30      40
inputs  -----GTCGACC---CACG---CGTCCG-TGGGTTTTCTTTTTTAATTATCCAAACAGT
              ..:::  ::::  :::::  :::  :::  :::  :::  :::  :::  :::
              CGGGATTACTGCCAGGCACAGCACGACCTCTATGCAGACAAGTG-AACTGTAGAACTGA
              10      20      30      40      50

              50      60      70
inputs  GGGCAGCT--TCCT-----CCCCCA-----CACCCAAGTATT----
              :::::  :::  :::::  :::  :::::
              TTACTGCTCCACCAAGAAGCCCCATAAGAGTGGTTATCCTGGACACAGAAGTGTTGAAT
              60      70      80      90      100     110

              80      90      100     110
inputs  TG-----CACAAATA-TTTGTGC--GGGGTATGG--GGGTGGGTTTTT---AAATC---
              ::  :::::  :::  :::  :::  :::  :::::  :::  :::  :::
              TGAATCCACAGAGCATTTTACAAGAGTTCTGACCTGGATGGGTAAACCTCAGTGCCT
              120     130     140     150     160     170

              120     130     140     150     160     170
inputs  TCGTTTCTCTTGGACA-----AGCACAGGGATCTCGTTCTCCT-CAT-TTTTGGGGGTGT
              ::  :::::  :::  :::  :::  :::  :::  :::  :::  :::
              TCTTTTCTGTTGGCCTCAGTATTACTGGATTGAAGAATTGCTGCTTCTTGTTAGGAGGTT
              180     190     200     210     220     230

              180     190     200
inputs  GTGGGGGACTTCTCAGGTCGTG-----TC---CCCA--GC-CT-----TCT-----
              .  ::::  :::  :::  :::  :::  :::  :::  :::
              CATTTCACTTATCATTACTTACAACCTTCACTCAAGCACTGAGAATTTCGAAGTGGAGT
              240     250     260     270     280     290

              210     220
inputs  -----CTGCAGTCCCTT--CTGCC--CTG-----
              ::  ::::  :::  :::  :::
              ATATTGAAGTAGACTTCAGTTTCTTTGCATCATTTCTGTATTCAATTTTTTTAATTATTT
              300     310     320     330     340     350

              230     240     250     260     270
inputs  CCGGGGCCC-GTCGGGAG-----GC-----GCCATGGCTCGGATGAACCGCCCGGCCCCCG
              :  ::::  :::  :::  :::  :::  :::  :::  :::  :::
              CATAACCCCTATTGAGTGTTTTTAATAAATAACATGGCTCGAATGAACCGCCAGCTCCT
              360     370     380     390     400     410

              280     290     300     310     320     330
inputs  GTGGAGGTGAGCTACAAACACATGCGCTTCTCTCATCACCACAACCCCAACGCCACG
              :::::  :::  :::::  :::  :::  :::  :::  :::  :::
              GTGGAAGTCACATACAAGAACATGAGATTCTTATTACACACAATCCAACCAATGCCACC
              420     430     440     450     460     470

              340     350     360     370     380     390
inputs  CTCAGCACCTTCATTGAGGACCTGAAGAAGTACGGGGCTACCACTGTGGTGCCTGTGTGT

```

FIGURE 4-1

TTAAACAAATTTATAGAGGAACCTTAAGAAGTATGGAGTTACCACAATAGTAAGAGTATGT  
480 490 500 510 520 530

inputs GAAGTGACCTATGACAAAACGCCGCTGGAGAAGGATGGCATCACCGTTGTGGACTGGCCG  
400 410 420 430 440 450  
:::: :::::::::: : : :::::::::::::: :: :: :: ::  
GAAGCAACTTATGACACTACTCTTGTGGAGAAAGAAGSTATCCATGTTCTTGATTGGCCT  
540 550 560 570 580 590

inputs TTTGACGATGGGCGCCCCCGCCGCGCAAGGTAGTGGAAGACTGGCTGAGCCTGGTGAAG  
460 470 480 490 500 510  
:::: ::::: ::::: :: . ::::: ::::: ::::: ::::: :: :::::  
TTTGATGATGGTGCACCACCATCCAACCAGATTGTTGATGACTGGTTAAGTCTTGTGAAA  
600 610 620 630 640 650

inputs GCCAAGTTCTGTGAGGCCCCCGGCAGCTGCGTGGCTGTGCCTGCGTGGCGGGCCTGGGC  
520 530 540 550 560 570  
. ::::: ::::: :: :: . : : : : : : : : : : : : : : : :  
ATTAAGTTTCGTGAAGAACCTGGTTGTTGATTGCTGTTCATTGCGTTGCAGGCCTTGGG  
660 670 680 690 700 710

inputs CGGGCTCCAGTCTTGTGGCGCTGGCCCTTATTGAGAGCGGGATGAAGTACGAGGACGCC  
580 590 600 610 620 630  
:  
AGAGCTCCAGTACTTGTGTCCTTAGCATTAAATTGAAGGTGGAATGAAATACGAAGATGCA  
720 730 740 750 760 770

inputs ATCCAGTTTCATCCGCCAGAAGCGCGCGGAGCCATCAACAGCAAGCAGCTCACCTACCTG  
640 650 660 670 680 690  
. :  
GTACAATTTCATAAGACAAAAGCGGCGTGGAGCTTTTAACAGCAAGCAAATTCTGTATTTG  
780 790 800 810 820 830

inputs GAGAAATACCGGCCCAAACAGAGGCTGCGGTTCAAAGA--CCCAC-----ACA-C  
700 710 720 730 740  
:  
GAGAAGTATCGTCCTAAAAATGCGGCTGCGTTTCAAAGATTCCAACGGTCATAGAAACAAC  
840 850 860 870 880 890

inputs ----GCA--CAAGA-----CCCGGTGCTGCGTTATGTAGCTC-----AGGAC  
750 760 770  
:  
TGTTGCATTCAATAAAATTGGGGTGCCTAATGCTACTGGAAGTGGAACCTTGAGATAGGGC  
900 910 920 930 940 950

inputs CTTGGCTG-----GGCCTG---GTCGTCAT-GTAGGTCAGGA-----CCTTG  
780 790 800 810  
:  
CTAATTTGTTATACATATTAGCCAACATGTTGGCTTAGTAAGTCTAATGAAGCTTCCATA  
960 970 980 990 1000 1010

inputs GCTGGACCTGGAGGC-----CCTG-CC-----CAGCC-CTG----  
820 830 840  
:  
GGAGTATTGAAAGGCAGTTTTACCAGGCCTCAAGCTAGACAGATTTGGCAACCTCTGTAT  
1020 1030 1040 1050 1060 1070

### FIGURE 4-2

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```

      850      860      870
inputs -----C--TCTGCCCA-----GCCCAGCAGGGGCTCCAGGC-----
      :  : : : : :  :  : : : : :  :  : :
      TTGGGTTACAGTCAACCTATTTGGATACTTGGCAAAGATTCTTGCTGTCAGCATATAAA
      1080      1090      1100      1110      1120      1130

      880      890
inputs -----CTTGGC-----TGGCC-----CCACATC--GC-----
      : : : : :  : : : :  : : : :  : :
      ATGTGCTTGTCATTTGTATCAATTGACCTTTCCCCAAATCATGCAGTATTGAGTTATGAC
      1140      1150      1160      1170      1180      1190

      900
inputs -----CTTTTCC-----TC-----
      : : : : :  : :
      TTGTTAAATCTATTCCCATGCCAGAATCTTCAATACATAAGAAATTTAGGAAGATTAG
      1200      1210      1220      1230      1240      1250

      910      920      930
inputs -----CCCGACAC--CT-----CCGTGCACTTGTG--TCCG
      : : : : :  : :  : : : : :  : :
      GTGCCAAATATCCAGCACAACTTGTATATTTTGTAGTACCATACAGAAGTAAATCCC
      1260      1270      1280      1290      1300      1310

      940      950
inputs AGGAGCGAGGAGC-----CCCTCGGGC-----CCTGGGT-
      : : : : :  : : : : :  : : : :
      AGGAACCTATGAACACTAGACCTTATGTGGTTTATTCCTTCAATCATTTCAAACATTGAAA
      1320      1330      1340      1350      1360      1370

      960      970      980      990
inputs ---GGCCT-C-TGG-----GCCCTTCTCCT-----GTCTCCGCCACTC---CC
      : : : : :  : : : :  : : : :  : : : :
      GTAGGGCTACATGGTTATTTGCCTGCTCACTTTATGTTTACATCTCCACATTCTATACC
      1380      1390      1400      1410      1420      1430

      1000      1010      1020      1030
inputs TCTG-GCGGC-----GCT-GGCCGTGG-----CTCTGTCTCTGTGAGG
      . . . : :  : : : : :  : : : : :  : : : : :
      AATATACGTCAGGTTTGCTTAACCATGATTTTTTTTTTTTTTACCAAGTCTTACAGTGA
      1440      1450      1460      1470      1480      1490

      1040      1050
inputs TGGGT---CGGGCG-CC-----CTC-----TGC-----CCGCC-----
      : . :  : : :  : :  : :  : :  : :
      TTATTTTACGTGTTTCCATGTATCTCACTTTGTGCTGTATTAAAAAACCTCCATTTTGA
      1500      1510      1520      1530      1540      1550

      1060      1070
inputs ---CCTC-----C-----CACA--C-----CAG-----CCAGGC---
      : . :  : :  : : : :  : :  : :
      AAATCTACGTTGTACAGAAGCACATGTCTTAAATGTCTTCAGACAAAAAGCCTTACATT
      1560      1570      1580      1590      1600      1610

      1080      1090      1100
inputs -----TGGTCTC-CTCTA-----GCCTGTTTGTGTG-GGGT

```

FIGURE 4-3

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```

      :: : : ::::
AATTTAATGTTTGCACCTCTGAGGTGCAACTTAACAGGGAGGGCCTGAGAAAAGAATGGGA
1620      1630      1640      1650      1660      1670

      1110      1120      1130
inputs GGGG-----TATATTT-----GTAACCA-----CTGG-GCCCCA-----
      ::::      :::::      :::::      :::::      :::::
GGGGGCTATTAATTATTTTGTAGCAAAATGTTGCCTTTGTCTTGTGCAAAACATGTAGAAT
1680      1690      1700      1710      1720      1730

      1140
inputs --GCC-----CT-----C
      :: :      ::
ATGCTCTTTAATCTAGTAAATATTTTTTTTAAAGGTAGAGATGCTTTGTTATTGTAATC
1740      1750      1760      1770      1780      1790

      1150      1160      1170
inputs TTTTGCACCC-----CTTGCTCTGA-----CGG-----CACC
      :::::      ::      :::::      ::      :::::      ::
ATAAACTTCCTGAAATCTTGTAAATTTTCCCATACTTATCAGAAGTGTGTTTACCAAC
1800      1810      1820      1830      1840      1850

      1180      1190
inputs TTAAATTA-TTAGA-----CC-CC-----
      :::::      :::::      ::      ::
TTATTTTGTGTTGAAAGTGTGATTTTTTTTTTCCCTTCCCAACCTCTCTTGCAAAAAAAGA
1860      1870      1880      1890      1900      1910

      1200      1210
inputs ---GG-----GCAG---TCAG-----GTGCT---
      ::      ::::      ::::
AATGGGTTTCTGCTAATGAATTGAGCAGAGATCTAATATTTTATATGCCTTTTGAGCTGT
1920      1930      1940      1950      1960      1970

inputs -----CCGGACA-----
      :      ::::
GTAAGTTAATATTTGATACTTGACAATTTGTTTTATTATGTAATTGATAAAATGGTGATG
1980      1990      2000      2010      2020      2030

      1220      1230      1240
inputs -----C--CCGAAGGCAATAA--AACAGGAG-----CC
      :      :::::      :::::
TGTATTAATGTTAGTTCAACCATATATTTATACTGTCTGGGGATGTGTGGTTATAGTTCT
2040      2050      2060      2070      2080      2090

      1250      1260      1270      1280
inputs GTGAAAAAAAAAAAAAAAA-----AAAAAAAAAAAAAAAAAAAAAAAA
      :::::      :::::      :::::      :::::
GTGGGAGAAATAATTTGTGAGTGTTCACCAGCTTGTAATACTAGTGCGAGAGCTGAA
2100      2110      2120      2130      2140      2150

      1290      1300      1310
inputs AAAAAAAAAAAAAAAAAAGGGCG-GC-----CGN-----
      :      :::::      :::::
ACATCTAAATAAATAATGACATGCATTTATCATCATTGAAA
2160      2170      2180      2190      2200

```

FIGURE 4-4

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```

ALIGN calculates a global alignment of two sequences
version 2.0uPlease cite: Myers and Miller, CABIOS (1989)
>PTPCAAXI
> fchrb003g03
scoring matrix: pam120.mat, gap penalties: -12/-4
78.6% identity; Global alignment score: 776

      10      20      30      40      50      60
inputs MARMNRPAPEVETVKMRFLITHNPTNATLNKFIIEELKKYGVTTIVRVCEATYDTTLVEK
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
      MARMNRPAPEVETVKMRFLITHNPTNATLNKFIIEELKKYGVTTIVRVCEATYDTTLVEK
      10      20      30      40      50      60

      70      80      90     100     110     120
inputs EGIHVDWPFDDGAPPSNQIVDDWLSLVKIKFREEPGCCIAVHCVAGLGRAPVLVALALI
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
      DGITVVDWPFDDGAPPSNQIVDDWLSLVKIKFREEPGCCIAVHCVAGLGRAPVLVALALI
      70      80      90     100     110     120

      130     140     150     160     170
inputs EGMKYEDAVQFIRQRRGAFNSKQLLYLEKYRPMRLRFKDSNGHRNNCCIQ
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
      EGMKYEDAVQFIRQRRGAFNSKQLLYLEKYRPMRLRFKDSNGHRNNCCIQ
      130     140     150     160     170

```

FIGURE 5

```
ALIGN calculates a global alignment of two sequences
version 2.0>Please cite: Myers and Miller, CABIOS (1989)
> b037d02 prot                                     263 aa vs.
> SwissProt P51452 - DUAL SPECIFICITY PROTEIN PHO   185 aa
scoring matrix: pam120.mat, gap penalties: -12/-4    .
22.5% identity;                               Global alignment score: 205

          10           20           30           40
inputs ----VDPRVRRL--LYTGKTAC----NHA-DEVWPGLYLGDDQDMANNRRELRRLGITHVL
      .  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :
MSGSFELSVQDLNDLLSDGSGCYSLPSQPCNEVTPRIYVGNASVAQDIPKLQKLGITHVL
          10           20           30           40           50           60

        50           60           70           80           90          100
inputs NASHSRW----RGTPEAYEGLGIRYLGVEAHDSPAFDMSIHFTAADFIHRALSQPGGKI
      ..  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :
NAAEGRSFMHVNTNANFYKDSGITYLGIKANDTQEFNL SAYFERAADFIDQALAQKNGRV
          70           80           90          100          110          120

       110          120          130          140          150          160
inputs LVHCAVGVSRSATLVLAYLMMLYHHLTLEAIKKVKDHRGEAEPAQTAVT-LCG-RGRGVRL
      :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :
LVHCREGYSRSPTLVIAYLMMRQKMDVKSALSIVRQNREIGPNDFLAQLCQLNDRLAKE
          130          140          150          160          170          180

       170          180          190          200          210          220
inputs GSVVDGHPPGRVDQGRRLGCRWKMLVGF EHRWDLARVLPGIHSFPFSLCPSVSLSPSPKT
      :  :
      GK LKP-----

       230          240          250          260
inputs KRAISAPALCAESQGYGKHECNVELCETPSIETDSEEMV

-----
```

### FIGURE 6

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```

ALIGN calculates a global alignment of two sequences
version 2.0uPlease cite: Myers and Miller, CABIOS (1989)
> Genbank 1657672 | Z68315 Caenorhabditis elegans 150 aa vs.
> Achrb067c02.aa 202 aa
scoring matrix: paml20.mat, gap penalties: -12/-4
24.0% identity; Global alignment score: -63

inputs -----MPFRSMKDEL
10
VRAATALLEAGLARVLFYPTLLYTLFRGKVPGRAHRDWHYRIDPTVLLGALPLRLSLTRQL
10 20 30 40 50 60
20 30 40 50 60 70
inputs IQENVGGVVCCTEEFELKAAMNAMREVVDWKNEGVFFAVPMKDFTGTAPRAEINEAVEF
.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.:
.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.:
VQDENVRGVITMNEEYETRFCLNSSQE--WKRLGVEQLRLSTVDMTGIPITLDNLQKGVQF
70 80 90 100 110
80 90 100 110 120 130
inputs IESVASKGKTVYVHCKAGRTRSATVATCYLMKSRNWMNSVWAEFLKDKRHQVLLRNAHWR
.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.: :.:
ALKYQSLGQCQVYVHCKAGRSRSATMVAAYLIQVHKWSPEEAVRAIAKIRSYIHIRPGQLD
120 130 140 150 160 170
140 150
inputs TVNEYRRFLDSNSSSTG-----SSN
..... :
VLKEFHKQITARATKDGTFVISKT
180 190 200

```

FIGURE 7

# Quantitative Results of cDNA Array

C2C12 Control		C2C12-CSAPTP-1Pooled	
<u>Genes</u>	<u>Density</u>	<u>Genes</u>	<u>Density</u>
	<u>Normalized to GAPDH</u>		<u>Normalized to GAPDH</u>
GAPDH	654	GAPDH	1068
PLA2	681	PLA2	1171
L1CAM	584	L1CAM	737
CD27	535	CD27	738
Caspase	780	Caspase	1920
Thymosin	847	Thymosin	1551
Hox7	494	Hox7	911
DB-1	2255	DB-1	522
			0.49

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FIGURE 8



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FIGURE 9

tgt tcc gct cag gca gag tcc tgc ccc tgc acc cac tcc ccc att ccc	48
Cys Ser Ala Gln Ala Glu Ser Cys Pro Cys Thr His Ser Pro Ile Pro	15
1	5
ggc ccc agg cca tgc ccc agg atg gac tca ctg cag aag cag gac ctc	96
Gly Pro Arg Pro Cys Pro Arg Met Asp Ser Leu Gln Lys Gln Asp Leu	30
20	25
cgg agg ccc aag atc cat ggg gca gtc cag gca tct ccc tac cag ccg	144
Arg Arg Pro Lys Ile His Gly Ala Val Gln Ala Ser Pro Tyr Gln Pro	45
35	40
ccc aca ttg gct tgc ctg cag cgc ttg ctg tgg gtc cgt cag gct gcc	192
Pro Thr Leu Ala Ser Leu Gln Arg Leu Leu Trp Val Arg Gln Ala Ala	60
50	55
aca ctg aac cat atc gat gag gtc tgg ccc agc ctc ttc ctg gga gat	240
Thr Leu Asn His Ile Asp Glu Val Trp Pro Ser Leu Phe Leu Gly Asp	80
65	70
gcg tac gca gcc cgg gac aag agc aag ctg atc cag ctg gga atc acc	288
Ala Tyr Ala Ala Arg Asp Lys Ser Lys Leu Ile Gln Leu Gly Ile Thr	95
85	90
cac gtt gtg aat gcc gct gca ggc aag ttc cag gtg gac aca ggt gcc	336
His Val Val Asn Ala Ala Ala Gly Lys Phe Gln Val Asp Thr Gly Ala	110
100	105
aaa ttc tac cgt gga atg tcc ctg gag tac tat ggc att gag gcg gac	384
Lys Phe Tyr Arg Gly Met Ser Leu Glu Tyr Tyr Gly Ile Glu Ala Asp	125
115	120
gac aac ccc ttc ttc gac ctc agt gtc tac ttt ctg cct gtt gct cga	432
Asp Asn Pro Phe Phe Asp Leu Ser Val Tyr Phe Leu Pro Val Ala Arg	140
130	135
tac atc cga gct gcc ctc agt gtt ccc caa ggc cgc gtg ctg gta cac	480
Tyr Ile Arg Ala Ala Leu Ser Val Pro Gln Gly Arg Val Leu Val His	160
145	150
tgt gcc atg ggg gta agc cgc tct gcc aca ctt gtc ctg gcc ttc ctc	528
Cys Ala Met Gly Val Ser Arg Ser Ala Thr Leu Val Leu Ala Phe Leu	175
165	170
atg atc tat gag aac atg acg ctg gta gag gcc atc cag acg gtg cag	576
Met Ile Tyr Glu Asn Met Thr Leu Val Glu Ala Ile Gln Thr Val Gln	190
180	185
gcc cac cgc aat atc tgc cct aac tca ggc ttc ctc cgg cag ctc cag	624
Ala His Arg Asn Ile Cys Pro Asn Ser Gly Phe Leu Arg Gln Leu Gln	205
195	200

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FIGURE 9, CONT.

```
gtt ctg gac aac cga ctg ggg cgg gac acg ggg cgg ttc tgatctggca 673
Val Leu Asp Asn Arg Leu Gly Arg Thr Gly Arg Phe
210
ggcagccagg atccctgacc cttggcccaa cccaccaccg cttggccctgg gaacagcagg 733
ctctgtgtgt tctagtgacc ctcagatgta aacagcaagt gggggctgag gcagagggcag 793
ggatagctgg gtggtgacct cttagcgggt ggatttcctt gacccaattc agagatttctt 853
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14/14

FIGURE 10

Symbol comparison table: /prod/ddm/seqanal/BLAST/matrix/aa/BLOSUM62  
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 Matrix made by matblas from blosum62.iij

Gap Weight: 12      Average Match: 2.778  
 Length Weight: 4      Average Mismatch: -2.248

Quality: 284      Length: 225  
 Ratio: 1.535      Gaps: 3  
 Percent Similarity: 50.276      Percent Identity: 41.989

Match display thresholds for the alignment(s):

| = IDENTITY  
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KPAa000fk x LFAa000fk      September 30, 1999 15:37 ..

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- 2 -

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- 14 -

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>7</sup> : <b>C12N 15/55, 9/16, 5/10, C07K 16/40, G01N 33/573, C12Q 1/68</b>		<b>A3</b>	(11) International Publication Number: <b>WO 00/18890</b>
			(43) International Publication Date: 6 April 2000 (06.04.00)
(21) International Application Number: <b>PCT/US99/22924</b>		(74) Agents: MANDRAGOURAS, Amy, E.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US) et al.	
(22) International Filing Date: 30 September 1999 (30.09.99)		(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: 09/164,193 30 September 1998 (30.09.98) US 09/163,833 30 September 1998 (30.09.98) US		Published <i>With international search report.</i>	
(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications US 09/164,193 (CIP) Filed on 30 September 1998 (30.09.98) US 09/163,833 (CIP) Filed on 30 September 1998 (30.09.98)		(88) Date of publication of the international search report: 28 September 2000 (28.09.00)	
(71) Applicant (for all designated States except US): MILLENNIUM PHARMACEUTICALS, INC. [US/US]; 75 Sidney Street, Cambridge, MA 02139 (US).			
(72) Inventor; and (75) Inventor/Applicant (for US only): ACTON, Susan [US/US]; Apartment No. 7, 90 Bynner Street, Jamaica Plain, MA 02130 (US).			
(54) Title: PROTEIN PHOSPHATASE MOLECULES AND USES THEREFOR			
(57) Abstract <p>CSAPTP (Cardiovascular System Associated Protein Tyrosine Phosphatase) polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated, full-length CSAPTP proteins, the invention further provides isolated CSAPTP fusion proteins, antigenic peptides and anti-CSAPTP antibodies. The invention also provides CSAPTP nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which a CSAPTP gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided</p>			

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DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

# INTERNATIONAL SEARCH REPORT

Internat. Application No  
PCT/US 99/22924

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 C12N15/55 C12N9/16 C12N5/10 C07K16/40 G01N33/573  
C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 14596 A (INCYTE PHARMA INC ;GOLI SURYA K (US)) 9 April 1998 (1998-04-09) abstract page 2, line 31 -page 3, line 3 page 20, line 12 -page 21, line 24 page 22, line 22 - line 32 seq id nos 3,4	1-3,5-22
Y	---	1-22
Y	WO 94 03611 A (MAX PLANCK GESELLSCHAFT) 17 February 1994 (1994-02-17) abstract page 40, line 29 -page 41, line 2 page 50, line 15 -page 51, line 13 ---	1-22
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

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- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*G\* document member of the same patent family

Date of the actual completion of the international search

8 March 2000

Date of mailing of the international search report

07.06.2000

Name and mailing address of the ISA

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NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

CEDER O.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/22924

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
1	<p>X ZENG ET AL.: "Homo sapiens potentially prenylated protein tyrosine phosphatase" EMBL SEQUENCE DATABASE, 10 August 1998 (1998-08-10), XP002132488 HEIDELBERG DE AC AF041434 the whole document</p> <p>P,X -&amp; ZENG ET AL.: "HPRL-3" EMBL SEQUENCE DATABASE, 1 November 1998 (1998-11-01), XP002132489 HEIDELBERG DE Ac 075365 the whole document</p>	1,2,8,9  8,9
1	<p>X ZENG ET AL.: "Protein tyrosine phosphatase 4A3 (MPRL-3)" EMBL SEQUENCE DATABASE, 1 August 1998 (1998-08-01), XP002132490 HEIDELBERG DE Ac 070275 the whole document</p> <p>A -&amp; ZENG ET AL.: "Mus musculus potentially prenylated protein tyrosine phosphatase mPRL-3 (Pr13) mRNA, complet compound" EMBL SEQUENCE DATABASE, 30 March 1998 (1998-03-30), XP002132491 HEIDELBERG DE Ac AF035645 the whole document &amp; ZENG ET AL.: "Mouse PRL-2 and PRL-3, two potentially prenylated protein tyrosine phosphatases homologous to PRL-1" BIOCHEM BIOPHYS RES COMMUN, vol. 244, 1998, pages 421-427, -----</p>	8,9  1,2,8,9

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 99/22924

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
  

Claims 1-22, as far as they relate to "the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_, \_\_\_\_, \_\_\_\_, or \_\_\_\_", could not be searched since the accession numbers were not disclosed, neither in the claims nor in the description.
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-22 all partly

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims 1-22, as far as they relate to "the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_.", could not be searched since the accession numbers were not disclosed, neither in the claims nor in the description.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.



FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-22 all partly

An isolated nucleic acid molecule, an isolated polypeptide, an antibody, a vector and a host cell and their uses all related to the CSAPTP sequences with SEQ ID NOS 1-3.

2. Claims: 1-22 all partly

An isolated nucleic acid molecule, an isolated polypeptide, an antibody, a vector and a host cell and their uses all related to the CSAPTP sequences with SEQ ID NOS 4-6.

3. Claims: 1-22 all partly

An isolated nucleic acid molecule, an isolated polypeptide, an antibody, a vector and a host cell and their uses all related to the CSAPTP sequences with SEQ ID NOS 7-9.

4. Claims: 1-22 all partly

An isolated nucleic acid molecule, an isolated polypeptide, an antibody, a vector and a host cell and their uses all related to the CSAPTP sequences with SEQ ID NOS 10-12.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat. Application No

PCT/US 99/22924

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9814596 A	09-04-1998	US 6020179 A	01-02-2000
		AU 4667397 A	24-04-1998
		EP 0951556 A	27-10-1999
WO 9403611 A	17-02-1994	AU 4707793 A	03-03-1994
		CA 2141847 A	17-02-1994
		CN 1091139 A	24-08-1994
		EP 0654083 A	24-05-1995
		JP 8501934 T	05-03-1996
		MX 9304769 A	31-05-1994
		US 5955592 A	21-09-1999
		US 5831009 A	03-11-1998

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(19) World Intellectual Property Organization  
International Bureau



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6 April 2000 (06.04.2000)

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(10) International Publication Number  
**WO 00/18890 A3**

(51) International Patent Classification<sup>7</sup>: **C12N 15/55**,  
9/16, 5/10, C07K 16/40, G01N 33/573, C12Q 1/68

[US/US]; Apartment No. 7, 90 Bynner Street, Jamaica  
Plain, MA 02130 (US).

(21) International Application Number: **PCT/US99/22924**

(74) Agents: **MANDRAGOURAS, Amy, E.**; Lahive & Cock-  
field, LLP, 28 State Street, Boston, MA 02109 et al. (US).

(22) International Filing Date:  
30 September 1999 (30.09.1999)

(81) Designated States (*national*): AU, CA, JP, US.

(25) Filing Language: English

(84) Designated States (*regional*): European patent (AT, BE,  
CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC,  
NL, PT, SE).

(26) Publication Language: English

(30) Priority Data:  
09/164,193 30 September 1998 (30.09.1998) US  
09/163,833 30 September 1998 (30.09.1998) US

Published:  
— With international search report.

(63) Related by continuation (CON) or continuation-in-part  
(CIP) to earlier applications:  
US 09/164,193 (CIP)  
Filed on 30 September 1998 (30.09.1998)  
US 09/163,833 (CIP)  
Filed on 30 September 1998 (30.09.1998)

(88) Date of publication of the international search report:  
28 September 2000

(48) Date of publication of this corrected version:  
1 February 2001

(71) Applicant (*for all designated States except US*): **MIL-  
LENNIUM PHARMACEUTICALS, INC.** [US/US]; 75  
Sidney Street, Cambridge, MA 02139 (US).

(15) Information about Correction:  
see PCT Gazette No. 05/2001 of 1 February 2001, Section  
II

(72) Inventor; and

(75) Inventor/Applicant (*for US only*): **ACTON, Susan**

*For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.*

(54) Title: **PROTEIN PHOSPHATASE MOLECULES AND USES THEREFOR**

(57) Abstract: CSAPTP (Cardiovascular System Associated Protein Tyrosine Phosphatase) polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated, full-length CSAPTP proteins, the invention further provides isolated CSAPTP fusion proteins, antigenic peptides and anti-CSAPTP antibodies. The invention also provides CSAPTP nucleic acid molecules, recombi-  
nant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which a CSAPTP gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.

WO 00/18890 A3

## NOVEL PROTEIN PHOSPHATASE MOLECULES AND USES THEREFOR

### Related Applications

This application claims priority to U.S. Patent Application Nos.: 09/164,193  
5 09/163,833 both filed on September 30, 1998, incorporated herein in their entirety by  
this reference.

### Background of the Invention

Phosphate tightly associated with protein has been known since the late  
10 nineteenth century. Since then, a variety of covalent linkages of phosphate to proteins  
have been found. The most common involve esterification of phosphate to serine and  
threonine, with smaller amounts being covalently linked to lysine, arginine, histidine,  
aspartic acid, glutamic acid, and cysteine. The occurrence of phosphorylated proteins  
implies the existence of one or more protein kinases capable of phosphorylating amino  
15 acid residues on proteins, and also of protein phosphatases capable of hydrolyzing  
phosphorylated amino acid residues on proteins.

Protein kinases play critical roles in the regulation of biochemical and  
morphological changes associated with cellular growth and division (D'Urso, G. et al.  
(1990) *Science* 250: 786-791; Birchmeier, C. et al. (1993) *Bioessays* 15: 185-189).  
20 They serve as growth factor receptors and signal transducers and have been implicated  
in cellular transformation and malignancy (Hunter, T. et al. (1992) *Cell* 70: 375-387;  
Posada, J. et al. (1992) *Mol. Biol. Cell* 3: 583-592; Hunter, T. et al. (1994) *Cell* 79: 573-  
582). For example, protein kinases have been shown to participate in the transmission  
of signals from growth-factor receptors (Sturgill, T. W. et al. (1988) *Nature* 344: 715-  
25 718; Gomez, N. et al. (1991) *Nature* 353: 170-173), control of entry of cells into mitosis  
(Nurse, P. (1990) *Nature* 344: 503-508; Maller, J. L. (1991) *Curr. Opin. Cell Biol.* 3:  
269-275) and regulation of actin bundling (Husain-Chishti, A. et al. (1988) *Nature* 334:  
718-721).

The overall level, in cells, of protein tyrosine phosphorylation, as well as the  
30 phosphorylated state of any given protein, arises from the balance of Protein Tyrosine  
Kinase (PTK) and Protein Tyrosine Phosphatase (PTPase) activities. Thus PTPases

have been proposed as key regulatory elements of cell growth control (Hunter, 1989, Cell 58:1013-1016).

PTKs were discovered and characterized more than one decade earlier than PTPases and in the last few years a large number of studies has led to the identification of many new PTPases and some of them have been accurately characterized. In addition, findings on the biological role of some PTPases in cells have recently been reported (Pondaven, 1991, *Adv Prot Phosphatases* 6:35-57). Current work suggests that PTKs and PTPases are equally important in many biological processes ranging from cell growth control to cell differentiation and development. In particular, the oncogenic potential of PTKs and the ability of PTPases to counteract PTK oncogenic activation by antiproliferative action suggests that the genes coding for PTPases, in many instances, may be considered tumor-suppressing genes or even anti-oncogenes.

The existence of PTPases was first predicted to explain the rapid loss of phosphorylation of *in vitro* phosphorylated membrane proteins (Carpenter *et al.*, 1979, *J Biol Chem* 254:4884-4891). The main PTPase in human placenta (PTP1B) was purified to homogeneity and sequenced (Tonks *et al.*, 1988, *J Biol Chem* 263:6722-2730; Charbonneau *et al.*, 1989, *PNAS USA* 86:5252-5256). Sequence homology between the catalytic domain of PTP1B and the leukocyte common antigen (LCA, or CD45) was demonstrated, indicating that PTPases can be considered a family of structurally related molecules.

The effects of many growth factors such as NGF, BDNF, NT3, FGF, insulin and IGF1 are known to be mediated by high-affinity receptors with tyrosine kinases activity (Fantl *et al.* *Annu. Rev. Biochem.*, 62 (1993) 453-481; Schlessinger and Ulrich *Neuron*, 9 (1992) 383-391; Ullrich and Schlessinger *Cell*, 61 (1990) 203-212). Expression of several tyrosine phosphatase genes has been detected in the brain (Jones *et al.* *J. Biol. Chem.*, 264 (1989) 7747-7753), including *RPTP $\alpha$*  (Kaplan *et al.* *Proc. Natl. Acad. Sci. USA*, 87 (1990) 7000-7004; Sap *et al.* *Proc. Natl. Acad. Sci. USA*, 87 (1990) 6112-6116), *RNPTPX* (Guan *et al.* *Proc Natl. Acad. Sci. USA*, 87 (1991) 1501-1505), *STEP* (Lombroso *et al.* *Proc. Natl. Acad. Sci. USA*, 88 (1991) 7242-7246), *SH-PTP2* (Freeman *et al.* *Proc. Natl. Acad. Sci. USA*, 89 (1992) 11239-11243), *MPTP $\delta$*  (Mizuno

*et al. Mol. Cell. Biol.*, 13 (1993) 5513-5523), *DPTP99A* and *DPTP10D* (Yang *et al. Cell*, 67 (1991) 661-673).

Intraventricular administration of either NGF, BDNF, insulin or IGF1 prevents delayed neuronal death in the CA1 subfield of the hippocampus (Beck *et al. J. Cereb Blood Flow Metab.*, 14 (1994) 689-692; Shigeno *et al. J. Neurosci.*, 11 (1991) 2914-2919; Zhu and Auer *J. Cereb. Blood Flow Metab.*, 14 (1994) 237-242).

Tyrosine kinase inhibitors block the tyrosine phosphorylation of MAP kinase (Blenis *Proc. Natl. Acad. Sci. USA*, 90 (1993) 5889-5892; Pelech and Sanghera *Science*, 257 (1992) 1335-1356) and prevent delayed neuronal death after forebrain ischemia (Kindy *J. Cereb. Blood Flow Metab.*, 13 (1993) 372-377). During reperfusion after ischemia, tyrosine phosphorylation of proteins increases in the hippocampus but some proteins in the hippocampus are dephosphorylated (Campos-Gonzalez *J. Neurochem.*, 59 (1992) 1955-1958; Hu and Wieloch *J. Neurochem.*, 62 (1994) 1357-1367; Takano *et al. J. Cereb. Blood Flow Metab.*, 15 (1995) 33-41). These observations suggest that tyrosine phosphorylation plays an important role in the delayed neuronal death which occurs as a result of ischemia-reperfusion injury.

A number of PTPases, in addition to the hydrolytic activity on phosphotyrosine, show some phosphoserine/phosphothreonine phosphatase activity. These enzymes, mostly localized in the nucleus and referred to as dual-specificity PTPases (dsPTPases), are emerging as a subclass of PTPases acting as important regulators of cell cycle control and mitogenic signal transduction possibly by controlling the activity of signal transduction proteins like ERK. In fact, they appear responsible for *in vivo* nuclear dephosphorylation and inactivation of nuclear dephosphorylation and inactivation of MAP kinases (Alessi *et al.*, 1995, *Curr Biol* 5:195-283). These enzymes exhibit sequence identity to the vaccinia H-1 gene product, the first identified dsPTPase (Guan *et al.*, 1991, *Nature* 350:359-362). Several dsPTPases differing from each other in length have been identified. These enzymes and the other PTPase subclasses share an active site sequence motif showing only a limited sequence homology beyond this region.

Given the importance of such protein tyrosine phosphatases in the regulation of the cell cycle, there exists a need to identify novel protein tyrosine phosphatases which

function as modulators in the cell cycle such as the suppression of proliferation and whose aberrant function can result in disorders arising from improper cell cycle regulation such as cancer.

## 5 Summary of the Invention

The present invention is based, at least in part, on the discovery of novel nucleic acid molecules and proteins encoded by such nucleic acid molecules, referred herein as "Cardiovascular System Associated Protein Tyrosine Phosphatase" ("CSAPTP") proteins. The CSAPTP nucleic acid and protein molecules of the present invention are  
10 useful as modulating agents in regulating a variety of cellular processes, e.g., cardiac cellular processes. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding CSAPTP proteins or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of CSAPTP-encoding nucleic acids.

15 In one embodiment, a CSAPTP nucleic acid molecule of the invention is at least 35%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more homologous to a nucleotide sequence (e.g., to the entire length of the nucleotide sequence) including SEQ ID NO:1, SEQ ID NO:3, or a complement thereof. In another embodiment, a CSAPTP nucleic acid molecule is at least 40%, 45%, 50%,  
20 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more homologous to a nucleotide sequence including SEQ ID NO:4, SEQ ID NO:6, or a complement thereof. In yet another embodiment, a CSAPTP nucleic acid molecule is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more homologous to a nucleotide sequence including SEQ ID NO:7, SEQ ID NO:9, or a complement thereof.  
25 In a further embodiment, a CSAPTP nucleic acid molecule is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more homologous to a nucleotide sequence including SEQ ID NO:10, SEQ ID NO:12, or a complement thereof.

In a preferred embodiment, the isolated nucleic acid molecule includes the  
30 nucleotide sequence shown SEQ ID NO:1 or 3, or a complement thereof. In another embodiment, the nucleic acid molecule includes SEQ ID NO:3 and nucleotides 1-248 of

SEQ ID NO:1. In another embodiment, the nucleic acid molecule includes SEQ ID NO:3 and nucleotides 768-1315 of SEQ ID NO:1. In another preferred embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:1 or 3. In another preferred embodiment, the nucleic acid molecule comprises a fragment of at least 994 nucleotides of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or a complement thereof.

In another preferred embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:4 or 6, or a complement thereof. In another embodiment, the nucleic acid molecule includes SEQ ID NO:6 and nucleotides 790-1016 of SEQ ID NO:4. In another preferred embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:4 or 6. In another preferred embodiment, the nucleic acid molecule comprises a fragment of at least 626 nucleotides of the nucleotide sequence of SEQ ID NO:4, SEQ ID NO:6, or a complement thereof.

In another preferred embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:7 or 9, or a complement thereof. In another embodiment, the nucleic acid molecule includes SEQ ID NO:9 and nucleotides 628-814 of SEQ ID NO:7. In another embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:7 or 9. In another preferred embodiment, the nucleic acid molecule comprises a fragment of at least 531 nucleotides of the nucleotide sequence of SEQ ID NO:7, SEQ ID NO:9, or a complement thereof.

In another preferred embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:10 or 12, or a complement thereof. In another embodiment, the nucleic acid molecule includes SEQ ID NO:12 and nucleotides 664-928 of SEQ ID NO:10. In another preferred embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:10 or 12. In another preferred embodiment, the nucleic acid molecule comprises a fragment of at least 241 nucleotides of the nucleotide sequence of SEQ ID NO:10, SEQ ID NO:12, or a complement thereof.

In another embodiment, a CSAPTP nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO. 11. In a preferred embodiment, a CSAPTP nucleic acid molecule includes a



nucleotide sequence encoding a protein having an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 78%, 79%, 80%, 85%, 90%, 95%, 99% or more homologous to an amino acid sequence including SEQ ID NO:2 (e.g., the entire amino acid sequence of SEQ ID NO:2). In another preferred embodiment, a CSAPTP nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 10%, 15%, 20%, 22%, 23%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more homologous to an amino acid sequence including SEQ ID NO:5 (e.g., the entire amino acid sequence of SEQ ID NO:5). In yet another preferred embodiment, a CSAPTP nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 10%, 15%, 20%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more homologous to an amino acid sequence including SEQ ID NO:8 (e.g., the entire amino acid sequence of SEQ ID NO:8). In another preferred embodiment, a CSAPTP nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 30%, 35%, 40%, 42%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to an amino acid sequence including SEQ ID NO:11 (e.g., the entire amino acid sequence of SEQ ID NO:11).

In another preferred embodiment, an isolated nucleic acid molecule encodes the amino acid sequence of a human CSAPTP. In yet another preferred embodiment, the nucleic acid molecule includes a nucleotide sequence encoding a protein which includes the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11. In yet another preferred embodiment, the nucleic acid molecule includes a nucleotide sequence encoding a protein having the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO: 8, or SEQ ID NO:11.

Another embodiment of the invention features nucleic acid molecules, preferably CSAPTP nucleic acid molecules, which specifically detect CSAPTP nucleic acid molecules relative to nucleic acid molecules encoding non-CSAPTP proteins. For example, in one embodiment, such a nucleic acid molecule is at least 240, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100 nucleotides in length and hybridizes under stringent conditions to a nucleic acid

molecule comprising the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, or a complement thereof.

In a particularly preferred embodiment, the nucleic acid molecule comprises a fragment of at least 994 nucleotides of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or a complement thereof. In preferred embodiments, the nucleic acid molecules are at least 15 (e.g., contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-17 and 1011-1315 of SEQ ID NO:1. In other preferred embodiments, the nucleic acid molecules comprise nucleotides 1-17 and 1011-1315 of SEQ ID NO:1. In other preferred embodiments, the nucleic acid molecules consist of nucleotides 1-17 and 1011-1315 of SEQ ID NO:1.

In another particularly preferred embodiment, the nucleic acid molecule comprises a fragment of at least 626 nucleotides of the nucleotide sequence of SEQ ID NO:4, SEQ ID NO:6, or a complement thereof. In preferred embodiments, the nucleic acid molecules are at least 15 (e.g., contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-342 of SEQ ID NO:4. In preferred embodiments, the nucleic acid molecules are at least 15 (e.g., contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1002-1016 of SEQ ID NO:4. In preferred embodiments, the nucleic acid molecules comprise nucleotides 1002-1016 of SEQ ID NO:4. In preferred embodiments, the nucleic acid molecules consist of nucleotides 1002-1016 of SEQ ID NO:4.

In another particularly preferred embodiment, the nucleic acid molecule comprises a fragment of at least 531 nucleotides of the nucleotide sequence of SEQ ID NO:7, SEQ ID NO:9, or a complement thereof. In preferred embodiments, the nucleic acid molecules are at least 15 (e.g., contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-103 and 774-814 of SEQ ID NO:7. In preferred embodiments, the nucleic acid molecules comprise nucleotides 1-103 and 774-814 of SEQ ID NO:7. In preferred embodiments, the nucleic acid molecules consist of nucleotides 1-103 and 774-814 of SEQ ID NO:7.

In another particularly preferred embodiment, the nucleic acid molecule comprises a fragment of at least 241 nucleotides of the nucleotide sequence of SEQ ID NO:10, SEQ ID NO:12, or a complement thereof. In preferred embodiments, the

nucleic acid molecules are at least 15 (e.g., contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-471 and 881-928 of SEQ ID NO:10. In other preferred embodiments, the nucleic acid molecules comprise nucleotides 1-471 and 881-928 of SEQ ID NO:1. In other preferred embodiments, the

5 nucleic acid molecules consist of nucleotides 1-471 and 881-928 of SEQ ID NO:1.

In other preferred embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide which includes the amino acid sequence of SEQ ID NO:2, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule which includes SEQ ID NO:1 or SEQ ID NO:3 under stringent conditions. In other

10 preferred embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide which includes the amino acid sequence of SEQ ID NO:5, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule which includes SEQ ID NO:4 or SEQ ID NO:6 under stringent conditions. In other preferred

15 embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide which includes the amino acid sequence of SEQ ID NO:8, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule which includes SEQ ID NO:7 or SEQ ID NO:9 under stringent conditions. In other preferred embodiments, the

20 nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide which includes the amino acid sequence of SEQ ID NO:11, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule which includes SEQ ID NO:10 or SEQ ID NO:12 under stringent conditions.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to a CSAPTP nucleic acid molecule, e.g., the coding strand of a CSAPTP nucleic acid molecule.

25 Another aspect of the invention provides a vector comprising a CSAPTP nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. The invention also provides a method for producing a protein, preferably a CSAPTP protein, by culturing in a suitable medium, a host cell, e.g., a mammalian host

30 cell such as a non-human mammalian cell, of the invention containing a recombinant expression vector, such that the protein is produced.

Another aspect of this invention features isolated or recombinant CSAPTP proteins and polypeptides. In one embodiment, the isolated protein, preferably a CSAPTP-1 protein, includes at least one CSAPTP-1 unique N-terminal domain and at least one phosphatase active domain. In another embodiment, the isolated protein, preferably a CSAPTP-1 protein, includes at least one CSAPTP-1 unique N-terminal domain and at least one phosphatase active domain and has an amino acid sequence which is at least 50%, 55%, 60%, 65%, 70%, 75%, 78%, 79%, 80%, 85%, 90%, 95%, 99% or more homologous to an amino acid sequence including SEQ ID NO:2. In yet another embodiment, the isolated protein, preferably a CSAPTP-1 protein, includes at least one CSAPTP-1 unique N-terminal domain and at least one phosphatase active domain and is expressed and/or functions in cells of the cardiovascular system. In an even further embodiment, the isolated protein, preferably a CSAPTP-1 protein, includes at least one CSAPTP-1 unique N-terminal domain and at least one phosphatase active domain and plays a role in signaling pathways associated with cellular growth, e.g., signaling pathways associated with cell cycle regulation. In another embodiment, the isolated protein, preferably a CSAPTP-1 protein, includes at least one CSAPTP-1 unique N-terminal domain and at least one phosphatase active domain and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3.

In another embodiment, the isolated protein, preferably a CSAPTP-2 protein, includes at least one CSAPTP-2 unique N-terminal domain and at least one phosphatase active domain. In another embodiment, the isolated protein, preferably a CSAPTP-2 protein, includes at least one CSAPTP-2 unique N-terminal domain and at least one phosphatase active domain and has an amino acid sequence which is at least 10%, 15%, 20%, 22%, 23%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more homologous to an amino acid sequence including SEQ ID NO:5. In yet another embodiment, the isolated protein, preferably a CSAPTP-2 protein, includes at least one CSAPTP-2 unique N-terminal domain and at least one phosphatase active domain and is expressed and/or functions in cells of the cardiovascular system. In an even further embodiment, the isolated protein, preferably a

CSAPTP-2 protein, includes at least one CSAPTP-2 unique N-terminal domain and at least one phosphatase active domain and plays a role in signaling pathways associated with cellular growth, e.g., signaling pathways associated with cell cycle regulation. In another embodiment, the isolated protein, preferably a CSAPTP-2 protein, includes at least one CSAPTP-2 unique N-terminal domain and at least one phosphatase active domain and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:4 or SEQ ID NO:6.

In yet another embodiment, the isolated protein, preferably a CSAPTP-3 protein, includes at least one CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain. In another embodiment, the isolated protein, preferably a CSAPTP-3 protein, includes at least one CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain and has an amino acid sequence which is at least 10%, 15%, 20%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more homologous to an amino acid sequence including SEQ ID NO:8. In yet another embodiment, the isolated protein, preferably a CSAPTP-3 protein, includes at least one CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain and is expressed and/or functions in cells of the cardiovascular system. In an even further embodiment, the isolated protein, preferably a CSAPTP-3 protein, includes at least one CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain and plays a role in signaling pathways associated with cellular growth, e.g., signaling pathways associated with cell cycle regulation. In another embodiment, the isolated protein, preferably a CSAPTP-3 protein, includes at least one CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:7 or SEQ ID NO:9.

In yet another embodiment, the isolated protein, preferably a CSAPTP-4 protein, includes at least one CSAPTP-4 unique N-terminal domain and at least one phosphatase active domain. In another embodiment, the isolated protein, preferably a CSAPTP-4 protein, includes at least one CSAPTP-4 unique N-terminal domain and at least one

phosphatase active domain and has an amino acid sequence which is at least 30%, 35%, 40%, 42%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or more homologous to an amino acid sequence including SEQ ID NO:11. In yet another embodiment, the isolated protein, preferably a CSAPTP-4 protein, includes at least one

5 CSAPTP-4 unique N-terminal domain and at least one phosphatase active domain and is expressed and/or functions in cells of the cardiovascular system. In an even further embodiment, the isolated protein, preferably a CSAPTP-4 protein, includes at least one CSAPTP-4 unique N-terminal domain and at least one phosphatase active domain and

10 plays a role in signaling pathways associated with cellular growth, e.g., signaling pathways associated with cell cycle regulation. In another embodiment, the isolated protein, preferably a CSAPTP-4 protein, includes at least one CSAPTP-4 unique N-terminal domain and at least one phosphatase active domain and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence

15 of SEQ ID NO:10 or SEQ ID NO:12.

In another embodiment, the isolated protein, preferably a CSAPTP protein, has an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11. In a preferred embodiment, the protein, preferably a CSAPTP protein, has an amino acid sequence at least 79%, 23%,

20 24%, 42% or more homologous to an amino acid sequence including SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, respectively (e.g., the entire amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11). In another embodiment, the invention features fragments of the proteins having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11,

25 wherein the fragment comprises at least 15 amino acids (e.g., contiguous amino acids) of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, respectively. In another embodiment, the protein, preferably a CSAPTP protein, has the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11.

30 Another embodiment of the invention features an isolated protein, preferably a CSAPTP protein, which is encoded by a nucleic acid molecule having a nucleotide

sequence at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more homologous to a nucleotide sequence (e.g., to the entire length of the nucleotide sequence) including SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:12, respectively, or  
5 a complement thereof. This invention further features an isolated protein, preferably a CSAPTP protein, which is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:12,  
10 or a complement thereof.

The proteins of the present invention or biologically active portions thereof, can be operatively linked to a non-CSAPTP polypeptide (e.g., heterologous amino acid sequences) to form fusion proteins. The invention further features antibodies, such as monoclonal or polyclonal antibodies, that specifically bind proteins of the invention,  
15 preferably CSAPTP proteins. In addition, the CSAPTP proteins or biologically active portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting the presence of a CSAPTP nucleic acid molecule, protein or polypeptide in a biological  
20 sample by contacting the biological sample with an agent capable of detecting a CSAPTP nucleic acid molecule, protein or polypeptide such that the presence of a CSAPTP nucleic acid molecule, protein or polypeptide is detected in the biological sample.

In another aspect, the present invention provides a method for detecting the  
25 presence of CSAPTP activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of CSAPTP activity such that the presence of CSAPTP activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating CSAPTP activity comprising contacting a cell capable of expressing CSAPTP with an agent that  
30 modulates CSAPTP activity such that CSAPTP activity in the cell is modulated. In one embodiment, the agent inhibits CSAPTP activity. In another embodiment, the agent

stimulates CSAPTP activity. In one embodiment, the agent is an antibody that specifically binds to a CSAPTP protein. In another embodiment, the agent modulates expression of CSAPTP by modulating transcription of a CSAPTP gene or translation of a CSAPTP mRNA. In yet another embodiment, the agent is a nucleic acid molecule  
5 having a nucleotide sequence that is antisense to the coding strand of a CSAPTP mRNA or a CSAPTP gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant CSAPTP protein or nucleic acid expression or activity by administering an agent which is a CSAPTP modulator to the  
10 subject. In one embodiment, the CSAPTP modulator is a CSAPTP protein. In another embodiment the CSAPTP modulator is a CSAPTP nucleic acid molecule. In yet another embodiment, the CSAPTP modulator is a peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the disorder characterized by aberrant CSAPTP protein or nucleic acid expression is an immune disorder, an anti-proliferative disorder, a  
15 proliferative disorder, e.g., cancer, for example sporadic cancers e.g., brain, breast and prostate; inherited autosomal-dominant cancer, e.g., Cowden's syndrome; renal and lung carcinomas; a metabolic disorder, e.g., diabetes, for example, impaired dephosphorylation of both the insulin receptor and insulin receptor substrate-1; viral pathogenesis, e.g., cancer, for example, adenovirus E1A-mediated cell proliferation; e.g.,  
20 Boubonic Plague, for example, pathogenic *Yersinia pestis* viral PTPase dephosphorylation of host phospho-proteins; a neural disorder; a cardiovascular disorder, e.g., congestive heart failure, or a disorder arising from improper dephosphorylation of phosphorylated protein.

The present invention also provides a diagnostic assay for identifying the  
25 presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a CSAPTP protein; (ii) mis-regulation of the gene; and (iii) aberrant post-translational modification of a CSAPTP protein, wherein a wild-type form of the gene encodes a protein with a CSAPTP activity.

In another aspect the invention provides a method for identifying a compound  
30 that binds to or modulates the activity of a CSAPTP protein, by providing an indicator composition comprising a CSAPTP protein having CSAPTP activity, contacting the



indicator composition with a test compound, and determining the effect of the test compound on CSAPTP activity in the indicator composition to identify a compound that modulates the activity of a CSAPTP protein.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

#### Brief Description of the Drawings

*Figure 1* depicts the cDNA sequence and predicted amino acid sequence of human CSAPTP-1. The nucleotide sequence corresponds to nucleic acids 1 to 1315 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 173 of SEQ ID NO:2. The coding region without the 5' and 3' untranslated regions of the human CSAPTP-1 gene is shown in SEQ ID NO:3.

*Figure 2* depicts the cDNA sequence and predicted amino acid sequence of human CSAPTP-2. The nucleotide sequence corresponds to nucleic acids 1 to 1016 of SEQ ID NO:4. The amino acid sequence corresponds to amino acids 1 to 263 of SEQ ID NO:5. The coding region without the 3' untranslated region of the human CSAPTP-2 gene is shown in SEQ ID NO:6.

*Figure 3* depicts the cDNA sequence and predicted amino acid sequence of human CSAPTP-3. The nucleotide sequence corresponds to nucleic acids 1 to 814 of SEQ ID NO:7. The amino acid sequence corresponds to amino acids 1 to 209 of SEQ ID NO:8. The coding region without the 3' untranslated region of the human CSAPTP-3 gene is shown in SEQ ID NO:9.

*Figure 4* depicts a global alignment between the CSAPTP-1 DNA sequence and the human protein tyrosine phosphatase DNA sequence (Accession No. AAB40597). This alignment were generated utilizing the ALIGN program with the following parameter setting: PAM120, gap penalties: -12/-4 (Myers, E. and Miller, W. (1988) "Optimal Alignments in Linear Space" CABIOS 4:11-17). The results showed a 38.7% identity between the two sequences.

Figure 5 depicts a global alignment between the CSAPTP-1 protein sequence and the human protein tyrosine phosphatase protein sequence (Accession No. AAB40597). This alignment were generated utilizing the ALIGN program with the following parameter setting: PAM120, gap penalties: -12/-4 (Myers, E. and Miller, W. (1988) "Optimal Alignments in Linear Space" CABIOS 4:11-17). The results showed a 78.6% identity between the two sequences.

Figure 6 depicts a global alignment between the CSAPTP-2 protein sequence and the human dual specificity phosphatase (SwissProt: P51452) protein sequence. This alignment were generated utilizing the ALIGN program with the following parameter setting: PAM120, gap penalties: -12/-4 (Myers, E. and Miller, W. (1988) "Optimal Alignments in Linear Space" CABIOS 4:11-17). The results showed a 22.5% identity between the two sequences.

Figure 7 depicts a global alignment between the CSAPTP-3 protein sequence and the SwissProt: Z68315 protein sequence. This alignment were generated utilizing the ALIGN program with the following parameter setting: PAM120, gap penalties: -12/-4 (Myers, E. and Miller, W. (1988) "Optimal Alignments in Linear Space" CABIOS 4:11-17). The results showed a 24% identity between the two sequences.

Figure 8 is a list of genes whose expression is altered by the expression of CSAPTP-1 in C2C12 cells.

Figure 9 depicts the cDNA sequence and predicted amino acid sequence of human CSAPTP-4. The nucleotide sequence corresponds to nucleic acids 1 to 912 of SEQ ID NO:10. The amino acid sequence corresponds to amino acids 1 to 221 of SEQ ID NO:11. The coding region without the 5' and 3' untranslated regions of the human CSAPTP-4 gene is shown in SEQ ID NO:12.

*Figure 10* depicts a global alignment between the human CSAPTP-4 amino acid sequence and dual specificity protein phosphatase (SwissProt No: P51452) amino acid sequence. This alignment were generated utilizing the ALIGN program with the following parameter setting: PAM120, gap penalties: -12/-4 (Myers, E. and Miller, W. (1988) "Optimal Alignments in Linear Space" CABIOS 4:11-17). The results showed a 42% identity between the two sequences.

### **Detailed Description of the Invention**

10       The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as "Cardiovascular System Associated Protein Tyrosine Phosphatase" or "CSAPTP" nucleic acid and polypeptide molecules, which play a role in or function in a variety of cellular processes, e.g., proliferation, differentiation, anti-proliferative mechanisms, immune responses, viral replication in a host, viral pathogenicity, neuroprotective responses, insulin responses, and cardiac cellular processes. In one embodiment, the CSAPTP molecules of the present invention modulate the activity of one or more proteins involved in a cellular proliferative and/or differentiative disorder, e.g., cancer. In another embodiment, the CSAPTP molecules of the present invention modulate the activity of one or more proteins involved in an immune cell disorder. In another embodiment, the CSAPTP molecules of the present invention modulate the activity of one or more proteins involved in viral replication and/or viral pathogenesis in a host. In another embodiment, the CSAPTP molecules of the present invention modulate the activity of one or more proteins involved in a neuroprotective response, e.g., neuronal response to ischemic injury. In another embodiment, the CSAPTP molecules of the present invention modulate the activity of one or more proteins involved in a metabolic disorder, e.g., diabetes. In one embodiment, the CSAPTP molecules of the present invention modulate the activity of one or more proteins involved in a cardiovascular disorder, e.g., congestive heart failure. In another embodiment, the CSAPTP molecules of the present invention are capable of modulating the phosphorylation state of a CSAPTP molecule or one or more proteins involved in cellular growth or differentiation, e.g., cardiac cell growth or differentiation.

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As used herein, the term "protein tyrosine phosphatase" or "PTPase" includes a protein or polypeptide (e.g., an enzyme) which is capable of facilitating (e.g., catalyzing) the removal of a phosphate group attached to a tyrosine, serine or threonine residue of a protein or polypeptides (e.g., a phosphoprotein). As referred to herein, a "protein tyrosine phosphatase" includes at least one catalytic domain having a specificity for dephosphorylating tyrosine residues or both serine/threonine residues and tyrosine residues (e.g., the dual specificity PTPases) and including at least about 200-400 amino acid residues in length, preferably about 200-300 amino acid residues in length, and more preferably about 250-300 amino acid residues in length. Phosphatases of the present invention preferably include a catalytic domain which includes at least one conserved motif or subdomain having at least about 30-50 amino acid residues, more preferably 40 amino acid residues which are conserved among PTPases. PTPases can be either soluble or membrane bound (see e.g., Brautigan *Biochem. biophys. Acta*, 1114 (1992) 63-77; Charbonneau *Ann. Rev. Cell Biol.*, 8 (1992) 463-493; Fisher *et al. Science*, 253 (1991) 401-406; Hunter *Cell*, 58 (1989) 1013-1016).

Membrane bound PTPases typically contain receptor-like extracellular regions connected to the intracellular (catalytic) domains by a short transmembrane segment (Streuli and Saito, 1993, *Adv Prot Phosphatases* 7:67-94). The non-transmembrane (cytoplasmic) PTPases typically include at least one catalytic domain (Koch *et al.*, 1991, *Science* 252:668-674).

As used herein, the term "protein kinase" includes a protein or polypeptide which is capable of modulating its own phosphorylation state or the phosphorylation state of another protein or polypeptide. Protein kinases can have a specificity for (i.e., a specificity to phosphorylate) serine/threonine residues, tyrosine residues, or both serine/threonine and tyrosine residues, e.g., the dual specificity kinases.

As used herein, the term "cardiovascular disorder" includes a disease, disorder, or state involving the cardiovascular system, e.g., the heart, the blood vessels, and/or the blood. A cardiovascular disorder can be caused by an imbalance in arterial pressure, a malfunction of the heart, or an occlusion of a blood vessel, e.g., by a thrombus. Examples of such disorders include hypertension, atherosclerosis, coronary artery spasm, coronary artery disease, valvular disease, arrhythmias, cardiomyopathies (e.g.,

dilated cardiomyopathy, idiopathic cardiomyopathy), arteriosclerosis, ischemia reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, atrial fibrillation, long-QT syndrome, congestive heart failure, sinus node disfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, myocardial infarction, cardiac hypertrophy, and coronary artery spasm.

As used herein, the term "congestive heart failure" includes a condition characterized by a diminished capacity of the heart to supply the oxygen demands of the body. Symptoms and signs of congestive heart failure include diminished blood flow to the various tissues of the body, accumulation of excess blood in the various organs, e.g., when the heart is unable to pump out the blood returned to it by the great veins, exertional dyspnea, fatigue, and/or peripheral edema, e.g., peripheral edema resulting from left ventricular dysfunction. Congestive heart failure may be acute or chronic. The manifestation of congestive heart failure usually occurs secondary to a variety of cardiac or systemic disorders that share a temporal or permanent loss of cardiac function. Examples of such disorders include hypertension, coronary artery disease, valvular disease, and cardiomyopathies, e.g., hypertrophic, dilative, or restrictive cardiomyopathies. Congestive heart failure is described in, for example, Cohn J.N. et al. (1998) *American Family Physician* 57:1901-04, the contents of which are incorporated herein by reference.

As used herein, the term "cardiac cellular processes" includes intra-cellular or inter-cellular processes involved in the functioning of the heart. Cellular processes involved in the nutrition and maintenance of the heart, the development of the heart, or the ability of the heart to pump blood to the rest of the body are intended to be covered by this term. Such processes include, for example, cardiac muscle contraction, distribution and transmission of electrical impulses, and cellular processes involved in the opening and closing of the cardiac valves. The term "cardiac cellular processes" further includes processes such as the transcription, translation and post- translational modification of proteins involved in the functioning of the heart, e.g., myofilament

specific proteins, such as troponin I, troponin T, myosin light chain 1 (MLC1), and  $\alpha$ -actinin.

As used herein, a "cellular proliferative disorder" includes a disorder, disease, or condition characterized by a deregulated, e.g., upregulated or downregulated, growth  
5 response. As used herein, a "cellular differentiative disorder" includes a disorder, disease, or condition characterized by aberrant or deficient cellular differentiation.

The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as CSAPTP protein and nucleic acid molecules, which  
10 comprise a family of molecules having certain conserved structural and functional features. The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide  
15 sequence homology as defined herein. Such family members can be naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologues of non-human origin. Members of a family may  
also have common functional characteristics.

One embodiment of the invention features CSAPTP nucleic acid molecules, preferably human CSAPTP molecules, e.g., CSAPTP-1, CSAPTP-2, CSAPTP-3 and  
20 CSAPTP-4, which were identified from cDNA libraries made from hearts of patients with congestive heart failure (CHF) of ischemic and idiopathic origin. The CSAPTP nucleic acid and protein molecules of the invention are described in further detail in the following subsections.

25 A. The CSAPTP-1 Nucleic Acid and Protein Molecules

One embodiment of the invention features CSAPTP-1 proteins and nucleic acid molecules which have sequence similarity with protein-tyrosine phosphatases. Accordingly, CSAPTP-1 polypeptides of the invention may interact with (e.g., bind to)  
30 at least one ligand which is a phosphorylated tyrosine of a protein and, thus, may be involved in the regulation of proliferation, anti-proliferative mechanisms, immune

responses, viral replication in a host, viral pathogenicity, neuroprotective responses, insulin responses, and cardiac cellular processes.

In one embodiment, the isolated proteins of the present invention, preferably CSAPTP-1 proteins, are identified based on the presence of a CSAPTP-1 unique N-terminal domain and at least one phosphatase active domain. As used herein, a "CSAPTP-1 unique N-terminal domain" includes a protein domain which is at least about 60-110 amino acid residues in length, preferably at least 60-100 amino acid residues in length, more preferably at least 60-90, or at least 60-80, or preferably 67 amino acid residues in length. In another embodiment, a CSAPTP-1 unique N-terminal domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a CSAPTP-1 unique N-terminal domain of a human CSAPTP-1 sequence set forth in SEQ ID NO:2 (e.g., amino acid residues 1-67 of the amino acid sequence as set forth in SEQ ID NO:2). As further defined herein, a CSAPTP-1 unique N-terminal domain of a CSAPTP-1 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-1 protein family. In a preferred embodiment, a CSAPTP-1 unique N-terminal domain has amino acid residues 1-67 of SEQ ID NO:2.

In another embodiment of the invention, a CSAPTP-1 family member is identified based on the presence of a phosphatase active domain. As used herein, a "phosphatase active domain" includes a protein domain which is at least 60-110 amino acid residues in length, preferably at least 70-110 amino acid residues in length, and more preferably at least 75-110, or at least 80-110, or preferably 105 amino acid residues in length, which is conserved in phosphatases which dephosphorylate tyrosine, serine, or threonine residues and is found in the catalytic domain of PTPases. A phosphatase active domain is capable of facilitating (e.g., catalyzing) the removal of a phosphate group attached to a tyrosine, serine or threonine residue of a protein (e.g., a phosphoprotein).

In one embodiment, a phosphatase active domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a phosphatase active domain of a human CSAPTP-1 sequence set forth in SEQ ID NO:2. In another embodiment, a phosphatase active domain has amino acid

residues 68-173 of the amino acid sequence as set forth in SEQ ID NO:2. Phosphatase active domains are described in, for example, Charbonneau, H. and Tonks, N.K. (1992) *Ann. Rev. Cell Biol.* 8:463-493, and Zhang, Z (1998) *Crit. Rev. Biochem. Mol. Biol.* 33(1):1-52, the contents of which are incorporated herein by reference. As further  
5 defined herein, a phosphatase active domain of a CSAPTP-1 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-1 protein family.

In another embodiment of the invention, a CSAPTP-1 family member is identified based on the presence of at least one phosphatase extended catalytic active  
10 domain within a phosphatase active domain. As used herein, a "phosphatase extended catalytic active domain" includes a protein domain which is at least 5-50 amino acid residues in length, preferably at least 10-40 amino acid residues in length, more preferably at least 15-25, or at least 20-22, or preferably 21 amino acid residues in length which is conserved in phosphatases which dephosphorylate tyrosine, serine, or threonine  
15 residues and found in the catalytic domain of PTPases. A phosphatase extended catalytic active domain is capable of facilitating (e.g., catalyzing) the removal of a phosphate group attached to a tyrosine, serine or threonine residue of a protein (e.g., a phosphoprotein). Preferably, the phosphatase extended catalytic active domain includes the following amino acid consensus sequence (VXVHCXAGXSRSTX (3) AYLM, X=  
20 any amino acid). Phosphatase extended catalytic active domains are described in, for example, Keyse, S.M. (1995) *Biochimica et Biophysica Acta* 1265:152-160, the contents of which are incorporated herein by reference. In another embodiment, a phosphatase extended catalytic active domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a  
25 phosphatase extended catalytic active domain of a human CSAPTP-1 sequence set forth in SEQ ID NO:2 (e.g., amino acid residues 100-120). In another embodiment, a phosphatase extended catalytic active domain has amino acid residues 100-120 of the amino acid sequence as set forth in SEQ ID NO:2. Phosphatase extended catalytic active domains are described in, for example, Ramponi G. and Stefani, M (1997) *Int. J.*  
30 *Biochem. Cell Biol.* 29(2):279-292, the contents of which are incorporated herein by reference. As further defined herein, a phosphatase extended catalytic active domain of



a CSAPTP-1 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-1 protein family.

In another embodiment of the invention, a CSAPTP-1 family member is identified based on the presence of at least one phosphatase catalytic active domain within a phosphatase active domain. As used herein, a "phosphatase catalytic active domain" includes a protein domain which is at least 5-20 amino acid residues in length, preferably at least 7-15 amino acid residues in length, and more preferably at least 8-12, or at least 9-11, or preferably 10 amino acid residues in length which is conserved in phosphatases which dephosphorylate tyrosine, serine, or threonine residues and found in the catalytic domain of PTPases. A phosphatase catalytic active domain is capable of facilitating (e.g., catalyzing) the removal of a phosphate group attached to a tyrosine, serine or threonine residue of a protein (e.g., a phosphoprotein). Preferably, in one embodiment, the phosphatase catalytic active domain includes the following amino acid consensus sequence ([I/V] HCXAGXXR [S/T] X= any amino acid). In another embodiment, a phosphatase catalytic active domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a phosphatase catalytic active domain of a human CSAPTP-1 sequence set forth in SEQ ID NO:2 (e.g., amino acid residues 102-111). Preferably, a phosphatase catalytic active domain has amino acid residues 102-111 of the amino acid sequence as set forth in SEQ ID NO:2. Phosphatase catalytic active domains are described in, for example, Keyse, S.M. (1995) *Biochimica et Biophysica Acta* 1265:152-160, the contents of which are incorporated herein by reference. As further defined herein, a phosphatase catalytic active domain of a CSAPTP-1 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-1 protein family.

In another embodiment of the invention, a CSAPTP-1 family member is identified based on the presence of at least one phosphatase catalytic core active domain within a phosphatase active domain. As used herein, a "phosphatase catalytic core active domain" includes a protein domain which is at least 5-15 amino acid residues in length, preferably at least 7-11, or at least 8-10, or preferably 9 amino acid residues in length

which is conserved in phosphatases which dephosphorylate tyrosine, serine, or threonine residues and found in the catalytic domain of PTPases. A phosphatase catalytic core active domain is capable of facilitating (e.g., catalyzing) the removal of a phosphate group attached to a tyrosine, serine or threonine residue of a protein (e.g., a phosphoprotein). Preferably, in one embodiment, the phosphatase catalytic core active domain includes the following amino acid consensus sequence (H/V)C(X)<sub>5</sub>R(S/T), X= any amino acid). In another embodiment, a phosphatase catalytic core active domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a phosphatase catalytic core active domain of a human CSAPTP-1 sequence set forth in SEQ ID NO:2 (e.g., amino acid residues 103-111). Preferably, a phosphatase catalytic core active domain has amino acid residues 103-111 of the amino acid sequence as set forth in SEQ ID NO:2. Phosphatase catalytic core active domains are described in, for example, Misra-Press, A. et al (1995) *J Biol. Chem.* 270(24):14587-14596, the contents of which are incorporated herein by reference. As further defined herein, a phosphatase catalytic core active domain of a CSAPTP-1 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-1 protein family.

In another embodiment of the invention, a CSAPTP-1 family member is identified based on the presence of at least one phosphatase catalytic invariant core active domain within the phosphatase active domain. As used herein, a "phosphatase catalytic invariant core active domain" includes a protein domain which is at least 4-15 amino acid residues in length, preferably at least 5-11 amino acid residues in length, and more preferably at least 6-10, or at least 8-9, or preferably 7 amino acid residues in length which is conserved in phosphatases which dephosphorylate tyrosine, serine, or threonine residues and found in the catalytic domain of PTPases. A phosphatase catalytic invariant core active domain is capable of facilitating (e.g., catalyzing) the removal of a phosphate group attached to a tyrosine, serine or threonine residue of a protein (e.g., a phosphoprotein). Preferably, the phosphatase catalytic invariant core active domain includes the following amino acid consensus sequence (C(X)<sub>5</sub>R, X= any amino acid). In another embodiment, a phosphatase catalytic invariant core active

domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a phosphatase catalytic invariant core active domain of a human CSAPTP-1 sequence set forth in SEQ ID NO:2 (e.g., amino acid residues 104-110). In another embodiment, a phosphatase catalytic invariant core active domain has amino acid residues 104-110 of the amino acid sequence as set forth in SEQ ID NO:2. Phosphatase catalytic invariant core active domains are described in, for example, Misra-Press, A. et al (1995) *J Biol. Chem.* 270(24):14587-14596, the contents of which are incorporated herein by reference. As further defined herein, a phosphatase catalytic invariant core active domain of a CSAPTP-1 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-1 protein family.

In another embodiment of the invention, a CSAPTP-1 family member is identified based on the presence of an intradomain disulfide bond consensus sequence. As used herein, an "intradomain disulfide bond consensus sequence" includes a protein domain which is at least 3-20 amino acid residues in length, preferably at least 3-15 amino acid residues in length, and more preferably at least 5-9, or at least 6-8, or preferably 7 amino acid residues in length, and is the site of cysteine-cysteine bonding within a single protein molecule. In one embodiment, an intradomain disulfide bond consensus sequence has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of an intradomain disulfide bond consensus sequence of a human CSAPTP-1 sequence set forth in SEQ ID NO:2 (e.g., amino acid residues 97-103). In another embodiment, an intradomain disulfide bond consensus sequence has amino acid residues 97-103 of the amino acid sequence as set forth in SEQ ID NO:2. Intradomain disulfide bond consensus sequence domains are described in, for example, Beck, S. and Barrel, B.G. (1988) *Nature* 331:269-272, the contents of which are incorporated herein by reference. As further defined herein, an intradomain disulfide bond consensus sequence of a CSAPTP-1 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-1 protein family.

- In another embodiment, CSAPTP-1 family members include at least 1, 2, or more Protein kinase C (PKC) phosphorylation sites. PKC phosphorylation sites can be found at least at residues 13-15 and 165-167 of SEQ ID NO:2. CSAPTP-1 family members can further include at least 1, 2, 3, 4 or more Casein kinase II phosphorylation sites. Casein kinase II phosphorylation sites can be found at least at residues 32-35, 56-59, 64-67 and 147-150 of SEQ ID NO:2. CSAPTP-1 family members can further include at least one tyrosine kinase phosphorylation site. Tyrosine kinase phosphorylation sites can be found at least at residues 47-53 of SEQ ID NO:2. CSAPTP-1 family members can further include at least 1, 2 or more N-glycosylation sites. N-glycosylation sites can be found at least at residues 27-30 of SEQ ID NO:2. CSAPTP-1 family members can further include at least 1, 2 or more N-myristoylation sites. N-myristoylation sites can be found at least at residues 97-102 and 139-144 of SEQ ID NO:2. CSAPTP-1 family members can further include at least 1, 2 or more homeobox domains. Homeobox domains can be found at least at residues 134-152 of SEQ ID NO:2.
- Isolated proteins of the present invention, preferably CSAPTP-1 proteins, have an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2 or are encoded by a nucleotide sequence which includes a nucleotide sequence sufficiently homologous to SEQ ID NO:1, SEQ ID NO:3. As used herein, the term "sufficiently homologous" includes a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30-40% homology, preferably 40-50% homology, more preferably 50-60%, and even more preferably 60-70%, 70-80%, or 80-90% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 30-40%, preferably 40-50%, more preferably 50-60%, 60-70%, 70-80%, or 80-90%

homology and share a common functional activity are defined herein as sufficiently homologous.

Accordingly, another embodiment of the invention features isolated CSATP-1 proteins and polypeptides having a CSATP-1 activity. Preferred proteins are CSATP-1 proteins having at least one CSATP-1 unique N-terminal domain and at least one PTPase active domain. Other preferred proteins are CSATP-1 proteins having at least a CSATP-1 unique N-terminal domain, at least one phosphatase active domain, and at least one phosphatase extended catalytic active domain. Additional preferred proteins are CSATP-1 proteins having at least a CSATP-1 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, and at least one phosphatase catalytic active domain. Yet other preferred proteins are CSATP-1 proteins having at least a CSATP-1 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, at least one phosphatase catalytic active domain, and at least one phosphatase catalytic core active domain. Other preferred proteins are CSATP-1 proteins having at least a CSATP-1 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, and at least one phosphatase catalytic active domain, at least one phosphatase catalytic core active domain, at least one phosphatase catalytic invariant core active domain. Further preferred proteins are CSATP-1 proteins having at least a CSATP-1 unique N-terminal domain, at least one phosphatase active domain, and at least one intradomain disulfide bond consensus sequence.

The nucleotide sequence of the isolated human CSATP-1 cDNA and the predicted amino acid sequence of the human CSPATP-1 polypeptide are shown in Figure 1 and in SEQ ID NOS:1, 3 and 2, respectively. A plasmid containing the nucleotide sequence encoding human CSATP-1 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on \_\_\_\_\_ and assigned Accession Number \_\_\_\_\_. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely

as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The CSAPTP-1 gene, which is approximately 1315 nucleotides in length, encodes a protein having a molecular weight of approximately 19 kD and which is approximately 173 amino acid residues in length. CSAPTP-1 message was detected in human heart and skeletal, in all rat tissues but predominantly in rat skeletal, heart, placenta, lung and brain.

In a preferred embodiment, CSAPTP-1 proteins of the invention have an amino acid sequence of about 50-100, more preferably about 100-150, and even more preferably about 150-200 or 173 amino acid residues in length.

B. The CSAPTP-2 Nucleic Acid and Protein Molecules

In another embodiment, isolated proteins of the present invention, preferably CSAPTP-2 proteins, are identified based on the presence of a CSAPTP-2 unique N-terminal domain and at least one phosphatase active domain as defined herein. As used herein, a "CSAPTP-2 unique N-terminal domain" includes a protein domain which is at least 110-130 amino acid residues in length, preferably at least 115-145 amino acid residues in length, more preferably at least 120-140, or at least 125-135, or preferably 131 amino acid residues in length. In another embodiment, a CSAPTP-2 unique N-terminal domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a CSAPTP-2 unique N-terminal domain of a human CSAPTP-2 sequence set forth in SEQ ID NO:5 (e.g., amino acid residues 1-131 of the amino acid sequence as set forth in SEQ ID NO:5). As further defined herein, a CSAPTP-2 unique N-terminal domain of a CSAPTP-2 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-2 protein family. Preferably, a "CSAPTP-2 unique N-terminal domain" has amino acid residues 1-131 of SEQ ID NO:5.

In yet another embodiment, the isolated CSAPTP-2 proteins are identified based on the presence of a CSAPTP-2 unique N-terminal domain, at least one phosphatase active domain, and at least one phosphatase extended catalytic active domain. In yet

another embodiment, the isolated CSATP-2 proteins are identified based on the presence of a CSATP-2 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain and at least one phosphatase catalytic active domain. In yet another embodiment, the isolated CSATP-  
5 2 proteins are identified based on the presence of a CSATP-2 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, at least one phosphatase catalytic active domain and at least one phosphatase catalytic core active domain. In yet another embodiment, the isolated CSATP-2 proteins are identified based on the presence of a CSATP-2 unique N-  
10 terminal domain and at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, at least one phosphatase catalytic active domain, at least one phosphatase catalytic core active domain, at least one phosphatase catalytic invariant core active domain, each of the above domains are described herein.

In a preferred embodiment, a CSATP-2 unique N-terminal domain includes  
15 amino acids 1-131. In another embodiment, a CSATP-2 phosphatase active domain includes amino acid residues 79-263. In another embodiment, a CSATP-2 phosphatase extended catalytic active domain includes amino acid residues 105-125. In another embodiment, a CSATP-2 phosphatase catalytic active domain includes amino acid residues 107-116. In another embodiment, a CSATP-2 phosphatase catalytic core  
20 active domain includes amino acid residues 108-116. In another embodiment, a CSATP-2 phosphatase catalytic invariant core active domain includes amino acid residues 109-115.

In another embodiment, CSATP-2 family members include at least 1, 2, 3 or more Protein kinase C (PKC) phosphorylation sites. PKC phosphorylation sites can be  
25 found at least at residues 11-13, 220-222 and 223-225 of SEQ ID NO:5. CSATP-2 family members can further include at least 1, 2, 3, 4 or more Casein kinase II phosphorylation sites. Casein kinase II phosphorylation sites can be found at least at residues 89-92, 131-134, 165-168 and 257-260 of SEQ ID NO:5. CSATP-2 family members can further include at least one amidation site. Amidation sites can be found at  
30 least at residues 176-179 of SEQ ID NO:5. CSATP-2 family members can further include at least 1 or more N-glycosylation sites. N-glycosylation sites can be found at

least at residues 50-53 of SEQ ID NO:5. CSAPTP-2 family members can further include at least 1, 2, 3, 4, 5 or more N-myristoylation sites. N-myristoylation sites can be found at least at residues 25-30, 58-63, 112-117, 160-165 and 246-251 of SEQ ID NO:5. CSAPTP-2 family members can further include at least 1, 2 or more helix-loop-helix DNA-binding domains. Helix-loop-helix DNA-binding domains can be found at least at residues 31-43 of SEQ ID NO:5.

Isolated proteins of the present invention, preferably CSAPTP-2 proteins, have an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:5 or are encoded by a nucleotide sequence which includes a nucleotide sequence sufficiently homologous to SEQ ID NO:4, SEQ ID NO:6. As used herein, the term "sufficiently homologous" includes a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30-40% homology, preferably 40-50% homology, more preferably 50-60%, and even more preferably 60-70%, 70-80%, or 80-90% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 30-40%, preferably 40-50%, more preferably 50-60%, 60-70%, 70-80%, or 80-90% homology and share a common functional activity are defined herein as sufficiently homologous.

Accordingly, another embodiment of the invention features isolated CSAPTP-2 proteins and polypeptides having a CSAPTP-2 activity as defined herein.

The nucleotide sequence of the isolated human CSAPTP-2 cDNA and the predicted amino acid sequence of the human CSPATP-2 polypeptide are shown in Figure 2 and in SEQ ID NOS:4,6 and 5, respectively. A plasmid containing the nucleotide sequence encoding human CSAPTP-2 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209,



on \_\_\_\_\_ and assigned Accession Number \_\_\_\_\_. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is  
5 required under 35 U.S.C. §112.

The CSAPTP-2 gene, which is approximately 1016 nucleotides in length, encodes a protein having a molecular weight of approximately 29 kD and which is approximately 263 amino acid residues in length. CSAPTP-2 message was detected in human with highest expression in skeletal muscle with some expression in heart and  
10 brain muscle, while in the rat expression was mainly found in skeletal muscle.

In a preferred embodiment, CSAPTP-2 proteins of the invention have an amino acid sequence of about 50-100, more preferably about 100-200, more preferably about 200-250, and even more preferably about 250-300 or 263 amino acid residues in length.

15 C. The CSAPTP-3 Nucleic Acid and Protein Molecules

In another embodiment, the isolated proteins of present invention, preferably CSAPTP-3 proteins, are identified based on the presence of a CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain. As used herein, a "CSAPTP-3 unique N-terminal domain" includes a protein domain which is at least 100-  
20 110 amino acid residues in length, preferably at least 95-115 amino acid residues in length, more preferably at least 90-120, or at least 85-125, or preferably 104 amino acid residues in length. In another embodiment, a CSAPTP-3 unique N-terminal domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a CSAPTP-3 unique N-terminal domain of a  
25 human CSAPTP-3 sequence set forth in SEQ ID NO:8 (e.g., amino acid residues 1-104 of the amino acid sequence as set forth in SEQ ID NO:8). As further defined herein, a CSAPTP-3 unique N-terminal domain of a CSAPTP-3 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-3 protein family. Preferably, a "CSAPTP-3  
30 unique N-terminal domain" has amino acid residues 1-104 of SEQ ID NO:8

Accordingly, another embodiment of the invention features isolated CSAPTP-3 proteins and polypeptides having a CSAPK-3 activity. Preferred proteins are CSAPTP-3 proteins having at least a CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain and, optionally, at least one intradomain disulfide bond consensus sequence. Other preferred proteins are CSAPTP-3 proteins having at least a CSAPTP-3 unique N-terminal domain and at least one phosphatase active domain, and at least one phosphatase extended catalytic active domain. Yet other preferred proteins are CSAPK-3 proteins having at least a CSAPTP-3 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, and at least one phosphatase catalytic active domain. Additionally, preferred proteins are CSAPTP-3 proteins having at least a CSAPTP-3 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, at least one phosphatase catalytic active domain, and at least one phosphatase catalytic core active domain. Yet other preferred proteins are CSAPTP-3 proteins having at least a CSAPTP-3 unique N-terminal domain, at least one phosphatase extended catalytic active domain, at least one phosphatase catalytic active domain, at least one phosphatase catalytic core active domain, and at least one phosphatase catalytic invariant core active domain.

The CSAPTP-3 unique N-terminal domain, the intradomain disulfide bond consensus sequence, the phosphatase active domain, the phosphatase extended catalytic active domain, the phosphatase catalytic active domain, the phosphatase catalytic core active domain, and the phosphatase catalytic invariant core active domain are described herein. In a preferred embodiment, a CSAPTP-3 unique N-terminal domain includes amino acid residues 1-104. In another embodiment, a CSAPTP-3 disulfide bond consensus sequence includes amino acid residues 131-137. In another embodiment, a CSAPTP-3 phosphatase active domain includes amino acid residues 105-209. In another embodiment, a CSAPTP-3 phosphatase extended catalytic active domain includes amino acid residues 134-154. In another embodiment, a CSAPTP-3 phosphatase catalytic active domain includes amino acid residues 136-145. In another embodiment, a CSAPTP-3 phosphatase catalytic core active domain includes amino acid

residues 137-145. In another embodiment, a CSAPTP-3 phosphatase catalytic invariant core active domain includes amino acid residues 138-144.

In another embodiment, CSAPTP-3 family members include at least 1, 2, 3 or more Protein kinase C (PKC) phosphorylation sites. PKC phosphorylation sites can be found at least at residues 22-24, 169-171 and 190-192 of SEQ ID NO:8. CSAPTP-3 family members can further include at least 1, 2, 3 or more Casein kinase II phosphorylation sites. Casein kinase II phosphorylation sites can be found at least at residues 76-79, 89-92 and 104-107 of SEQ ID NO:8. CSAPTP-3 family members can further include at least one cAMP and cGMP dependent protein kinase phosphorylation site. cAMP and cGMP dependent protein kinase phosphorylation sites can be found at least at residues 24-27 of SEQ ID NO:8. CSAPTP-3 family members can further include at least one or more N-glycosylation sites. N-glycosylation sites can be found at least at residues 88-91 of SEQ ID NO:8. CSAPTP-3 family members can further include at least 1, 2, 3, 4 or more N-myristoylation sites. N-myristoylation sites can be found at least at residues 19-24, 41-46 and 120-125 of SEQ ID NO:8. CSAPTP-3 family members can further include at least 1, 2 or more transcription factor TFIIB repeats. Transcription factor TFIIB repeats can be found at least at residues 108-179 of SEQ ID NO:8.

Isolated proteins of the present invention, preferably CSAPTP-3 proteins, have an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:8 or are encoded by a nucleotide sequence which includes a nucleotide sequence sufficiently homologous to SEQ ID NO:7, SEQ ID NO:9. As used herein, the term "sufficiently homologous" includes a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30-40% homology, preferably 40-50% homology, more preferably 50-60%, and even more preferably 60-70%, 70-80%, or 80-90% homology across the amino acid sequences of the domains and contain at least one

and preferably two structural domains or motifs, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 30-40%, preferably 40-50%, more preferably 50-60%, 60-70%, 70-80%, or 80-90% homology and share a common functional activity are defined herein as sufficiently  
5 homologous.

The nucleotide sequence of the isolated human CSATP-3 cDNA and the predicted amino acid sequence of the human CSPATP-3 polypeptide are shown in Figure 3 and in SEQ ID NOS:7,9 and 8, respectively. A plasmid containing the nucleotide sequence encoding human CSATP-3 was deposited with American Type  
10 Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on \_\_\_\_\_ and assigned Accession Number \_\_\_\_\_. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is  
15 required under 35 U.S.C. §112.

The CSATP-3 gene, which is approximately 814 nucleotides in length, encodes a protein having a molecular weight of approximately 23 kD and which is approximately 209 amino acid residues in length. CSATP-3 message was detected in human in highest in heart and skeletal and also found in brain, placenta, kidney and pancreas.

20 In a preferred embodiment, CSATP-3 proteins of the invention have an amino acid sequence of about 50-100, more preferably about 100-200, and even more preferably about 200-250 or 209 amino acid residues in length.

#### D. The CSATP-4 Nucleic Acid and Protein Molecules

25 In another embodiment, the isolated proteins of present invention, preferably CSATP-4 proteins, are identified based on the presence of a CSATP-4 unique N-terminal domain and at least one phosphatase active domain. As used herein, a "CSATP-4 unique N-terminal domain" includes a protein domain which is at least 100-200 amino acid residues in length, preferably at least 110-190 amino acid residues in  
30 length, more preferably at least 120-180, or at least 130-170, or preferably about 158 amino acid residues in length. In another embodiment, a CSATP-4 unique N-terminal

domain has at least 65-95%, preferably at least 70-90%, and more preferably at least 75-85% homology with the amino acid sequence of a CSAPTP-4 unique N-terminal domain of a human CSAPTP-4 sequence set forth in SEQ ID NO:11 (e.g., amino acid residues 1-158 of the amino acid sequence as set forth in SEQ ID NO:11). As further defined

5 herein, a CSAPTP-4 unique N-terminal domain of a CSAPTP-4 protein family member, however, is not sufficiently homologous to the amino acid sequence of a member of another protein family, such as a non-CSAPTP-4 protein. Preferably, a "CSAPTP-4 unique N-terminal domain" has amino acid residues 1-158 of SEQ ID NO:11

Accordingly, another embodiment of the invention features isolated CSAPTP-4

10 proteins and polypeptides having a CSAPK-4 activity. Preferred proteins are CSAPTP-4 proteins having at least a CSAPTP-4 unique N-terminal domain and at least one phosphatase active domain. Other preferred proteins are CSAPTP-4 proteins having at least a CSAPTP-4 unique N-terminal domain and at least one phosphatase active domain, and at least one phosphatase extended catalytic active domain. Yet other

15 preferred proteins are CSAPK-4 proteins having at least a CSAPTP-4 unique N-terminal domain, at least one phosphatase active domain, at least one phosphatase extended catalytic active domain, and at least one phosphatase catalytic active domain. Additionally, preferred proteins are CSAPTP-4 proteins having at least a CSAPTP-4 unique N-terminal domain, at least one phosphatase active domain, at least one

20 phosphatase extended catalytic active domain, at least one phosphatase catalytic active domain, and at least one phosphatase catalytic core active domain. Yet other preferred proteins are CSAPTP-4 proteins having at least a CSAPTP-4 unique N-terminal domain, at least one phosphatase extended catalytic active domain, at least one phosphatase catalytic active domain, at least one phosphatase catalytic core active domain, and at

25 least one phosphatase catalytic invariant core active domain.

The CSAPTP-4 unique N-terminal domain, the phosphatase active domain, the phosphatase extended catalytic active domain, the phosphatase catalytic active domain, the phosphatase catalytic core active domain, and the phosphatase catalytic invariant core active domain are described herein. In one embodiment, a CSAPTP-4 phosphatase

30 active domain includes amino acid residues 68-221 of SEQ ID NO:11. In another embodiment, a CSAPTP-4 phosphatase extended catalytic active domain includes amino

acid residues 159-171 of SEQ ID NO:11. In another embodiment, a CSAPTP-4 phosphatase catalytic active domain includes amino acid residues 159-168 of SEQ ID NO:11. In another embodiment, a CSAPTP-4 phosphatase catalytic core active domain includes amino acid residues 160-168 of SEQ ID NO:11. In another embodiment, a

5 CSAPTP-3 phosphatase catalytic invariant core active domain includes amino acid residues 161-167 of SEQ ID NO:11.

In another embodiment, CSAPTP-4 family members include at least one Protein kinase C (PKC) phosphorylation site. PKC phosphorylation sites can be found at least at residues 218-220 of SEQ ID NO:11. CSAPTP-4 family members can further include

10 at least one Casein kinase II phosphorylation site. Casein kinase II phosphorylation sites can be found at least at residues 183-186 of SEQ ID NO:11. CSAPTP-4 family members can further include at least one N-glycosylation site. N-glycosylation sites can be found at least at residues 181-184 of SEQ ID NO:11. CSAPTP-4 family members can further include at least one N-myristoylation site. N-myristoylation sites can be

15 found at least at residues 39-44 and 164-169 of SEQ ID NO:11.

Isolated proteins of the present invention, preferably CSAPTP-4 proteins, have an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:11 or are encoded by a nucleotide sequence which includes a nucleotide sequence sufficiently homologous to SEQ ID NO:10, SEQ ID NO:12. As used herein, the term

20 "sufficiently homologous" includes a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common

25 functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30-40% homology, preferably 40-50% homology, more preferably 50-60%, and even more preferably 60-70%, 70-80%, or 80-90% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently

30 homologous. Furthermore, amino acid or nucleotide sequences which share at least 30-40%, preferably 40-50%, more preferably 50-60%, 60-70%, 70-80%, or 80-90%

homology and share a common functional activity are defined herein as sufficiently homologous.

The nucleotide sequence of the isolated human CSATP-4 cDNA and the predicted amino acid sequence of the human CSPATP-4 polypeptide are shown in Figure 9 and in SEQ ID NOS:10, 12 and 11, respectively. A plasmid containing the nucleotide sequence encoding human CSATP-3 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on \_\_\_\_\_ and assigned Accession Number \_\_\_\_\_. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The CSATP-4 gene, which is approximately 928 nucleotides in length, encodes a protein having a molecular weight of approximately 24 kD and which is approximately 221 amino acid residues in length. CSATP-4 message was detected in human heart and skeletal muscle.

In another preferred embodiment, CSATP-4 nucleic acids of the invention include at least 100 consecutive nucleotides, more preferably at least 200 consecutive nucleotides, more preferably at least 400 consecutive nucleotides, more preferably at least 600 consecutive nucleotides, more preferably at least 800 consecutive nucleotides, more preferably at least 900 consecutive nucleotides of the nucleotide sequence shown SEQ ID NO:10 or 12, or a complement thereof.

In another preferred embodiment, CSATP-4 proteins of the invention include at least 50 consecutive amino acids, more preferably at least 100 consecutive amino acids, more preferably at least 150 consecutive amino acids, more preferably at least 200 consecutive amino acids of the amino acid sequence shown SEQ ID NO:11.

#### E. CSATP Activities

As used interchangeably herein, a "CSATP activity", "biological activity of CSATP" or "functional activity of CSATP", includes an activity exerted by a CSATP protein, polypeptide or nucleic acid molecule as determined *in vivo*, *in vitro*, or

*in situ*, according to standard techniques. In one embodiment, a CSAPTP activity is a direct activity, such as an association with a CSAPTP-target molecule. As used herein, a "target molecule" is a molecule with which a CSAPTP protein binds or interacts in nature, such that CSAPTP-mediated function is achieved. A CSAPTP target molecule  
5 can be a CSAPTP protein or polypeptide of the present invention or a non-CSAPTP molecule. For example, a CSAPTP target molecule can be a non-CSAPTP protein molecule. Alternatively, a CSAPTP activity is an indirect activity, such as an activity mediated by interaction of the CSAPTP protein with a CSAPTP target molecule such that the target molecule modulates a downstream cellular activity (e.g., interaction of an  
10 CSAPTP molecule with a CSAPTP target molecule can modulate the activity of that target molecule on an immune cell).

In a preferred embodiment, a CSAPTP activity is at least one or more of the following activities: (i) interaction of a CSAPTP protein with a CSAPTP target molecule; (ii) interaction of a CSAPTP protein with a CSAPTP target molecule,  
15 wherein the CSAPTP target is a ligand, e.g., phosphorylated amino acid residue of a phosphorylated protein (e.g., a phosphatase, for example, a cell cycle regulatory phosphatase, e.g., Cdc25A phosphatase, a cell cycle phosphatase which regulates the G1/S-phase transition, a CSAPTPase), a kinase (e.g., Mitogen Activating Protein (MAP) kinase, or a Cardiovascular associated Protein Tyrosine Kinase (CSAPTK)); (iii)  
20 interaction of a CSAPTP protein with a CSAPTP target molecule, wherein the CSAPTP target is a receptor, e.g., insulin receptor, insulin receptor substrate 1; (iv) interaction of a CSAPTP protein with a CSAPTP target molecule, wherein the CSAPTP target is a viral protein, e.g., *vaccinia* viral transcription-mediating proteins, Myxoma viral proteins, Shope Fibroma viral proteins, *Leishmania donovani*, *Trypanosoma brucei* and  
25 *Trypanosoma cruzi* viral proteins.

In yet another preferred embodiment, a CSAPTP activity is at least one or more of the following activities: (1) regulation of cell cycle, e.g., dephosphorylation of phosphorylated proteins involved in the cell cycle, e.g., dephosphorylation of phosphorylated proteins involved in the cell cycle, e.g., proliferation-mediating proteins,  
30 e.g., Cdc25A, a cell cycle phosphatase which regulates the G1/S-phase transition, e.g., anti-proliferative proteins, either *in vitro*, *in vivo* or *in situ*; (2) mediation of the viral



pathogenicity, e.g., viral phosphatase mediated dephosphorylation of host phosphorylated proteins, for example, viral induced disease, e.g., *Yersinia* pathogenesis, for example, *Yersinia pestis* (Bubonic Plague), e.g., viral phosphatase mediated dephosphorylation of host anti-proliferative phosphorylated proteins, for example, viral induced proliferative diseases, e.g., viral induced cancers, either *in vitro*, *in vivo* or *in situ*; (3) regulation of the phosphorylation state of receptors, e.g., insulin receptor, e.g., insulin receptor substrate 1, either *in vitro*, *in vivo* or *in situ*.

Various aspects of the invention are described in further detail in the following subsections:

#### I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode CSAPTP proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify CSAPTP-encoding nucleic acids (e.g., CSAPTP mRNA) and fragments for use as PCR primers for the amplification or mutation of CSAPTP nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated CSAPTP nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by

recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. For example, using all or portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, as a hybridization probe, CSAPTP nucleic acid molecules can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_ can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to CSAPTP nucleotide sequences can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:10. The sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:10, corresponds to the human CSAPTP-1, CSAPTP-2 CSAPTP-3 and CSAPTP-4 cDNAs, respectively. These cDNAs comprise sequence encoding the human CSAPTP-1, CSAPTP-2, CSAPTP-3 and CSAPTP-4 proteins, i.e., "the coding region", from nucleotides 247-767, 1-789, 1-627 and 1-663 respectively, as well as 5' untranslated and 3' untranslated sequences. Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:10 (e.g., nucleotides 247-767, 1-789, 1-627 and 1-663, respectively), corresponding to SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, and SEQ ID NO:12, respectively).

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO. 12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_ or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 12, respectively, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 12, respectively, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least 30, 31, 32-35%, preferably about 35-36%, 36-40%, more preferably at least 40-43%, 43-45%, more preferably at least 45-50%, and even more preferably at least 50-55%, 55-57%, 57-60%,  
5 60-65%, 65-70%, 70-75%, 75-80%, 80-85%, 85-90%, or 90-95% or more homologous to the nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, or a portion of any of these  
10 nucleotide sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence (e.g., to the entire length of the nucleotide sequence) of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 12, or the nucleotide sequence of the DNA insert  
15 of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of a CSAPTP protein. The nucleotide sequence determined from the cloning of the CSAPTP gene allows for the generation of probes and primers designed for use in identifying and/or cloning other CSAPTP family  
20 members, as well as CSAPTP homologues from other species. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, SEQ ID  
25 NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, of an anti-sense sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 12, or the nucleotide sequence of the DNA insert  
30 of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, or of a naturally occurring mutant of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ

ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_.

5 In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is about 100, preferably 100-200, preferably 200-300, more preferably 300-400, more preferably 400-500, and even more preferably 500-516 nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_.

10 In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is about 450, preferably 450-550, more preferably 550-650, more preferably 650-750, and even more preferably 750-789 nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA  
15 insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_.

In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is about 300, preferably 300-400, more preferably 400-500, more preferably 500-600, and even more preferably 600-627 nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic  
20 acid molecule of SEQ ID NO:7, SEQ ID NO:9, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_.

In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is about 200, preferably 200-300, more preferably 300-400, more preferably 400-500, and even more preferably 500-600 or  
25 more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_.

Probes based on the CSAPTP nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In  
30 preferred embodiments, the probe further comprises a label group attached thereto, e.g., the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme

co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a CSAPTP protein, such as by measuring a level of a CSAPTP-encoding nucleic acid in a sample of cells from a subject e.g., detecting CSAPTP mRNA levels or determining whether a genomic CSAPTP gene has been

5 mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of a CSAPTP protein" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid

10 deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, which encodes a polypeptide having a CSAPTP biological activity (the biological activities of the CSAPTP proteins have previously been described), expressing the encoded portion of the CSAPTP protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the CSAPTP protein.

15 The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, due to degeneracy of the genetic code and thus encode the

20 same CSAPTP proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide

25 sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO: 11.

In addition to the CSAPTP nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited

30 with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the

amino acid sequences of the CSAPTP proteins may exist within a population (e.g., the human population). Such genetic polymorphism in the CSAPTP genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open  
5 reading frame encoding a CSAPTP protein, preferably a mammalian CSAPTP protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of a CSAPTP gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in CSAPTP genes that are the result of natural allelic variation and that do not alter the functional activity of a CSAPTP protein are intended  
10 to be within the scope of the invention.

Moreover, nucleic acid molecules encoding other CSAPTP family members, and thus which have a nucleotide sequence which differs from the CSAPTP sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of  
15 the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_ are intended to be within the scope of the invention. For example, a CSAPTP cDNA can be identified based on the nucleotide sequence of human CSAPTP. Moreover, nucleic acid molecules encoding CSAPTP proteins from different species, and thus which have a nucleotide sequence which differs from the CSAPTP sequences of SEQ ID NO:1, SEQ  
20 ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_ are intended to be within the scope of the invention. For example, an mouse CSAPTP cDNA can be identified based on the nucleotide sequence of a human CSAPTP.

25 Nucleic acid molecules corresponding to natural allelic variants and homologues of the CSAPTP cDNAs of the invention can be isolated based on their homology to the CSAPTP nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, 5 SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_. In other embodiment, the nucleic acid is at least 30, 50, 100, 250, 500, 750, 1000, 1500 nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 10 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more homologous to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least 70%, more preferably at least 80%, even more preferably at least 85% or 90% homologous to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art 15 and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions 20 to the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, or SEQ ID NO:12, corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule includes an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

25 In addition to naturally-occurring allelic variants of the CSAPTP sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with 30 ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, thereby leading to changes in the amino acid sequence of the encoded CSAPTP proteins, without altering the functional



ability of the CSAPTP proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of CSAPTP (e.g., the sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the CSAPTP proteins of the present invention, are predicted to be particularly unamenable to alteration (e.g., the ten conserved cysteines involved in forming disulfide linkages or the conserved histidine, aspartate, or serine of the active enzymatic site). Moreover, amino acid residues that are defined by the CSAPTP intradomain disulfide bond consensus sequence, the phosphatase active domain, the phosphatase extended catalytic active domain, the phosphatase catalytic active domain, the phosphatase catalytic core active domain, and the phosphatase catalytic invariant core active domain are signature sequences and are therefore particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the CSAPTP proteins of the present invention and other members of the Protein Tyrosine Phosphatase superfamily or protein families containing tyrosine, serine or threonine phosphatase activity are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding CSAPTP proteins that contain changes in amino acid residues that are not essential for activity. Such CSAPTP proteins differ in amino acid sequence from SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11 yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least 10%, 15%, 20%, 23%, 24%, 25%, 30%, 35%, 40%, 42%, 45%, 50%, 55%, 57%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11. Preferably, the protein encoded by the nucleic acid molecule is at least 65-70% homologous to SEQ ID

NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, more preferably at least 75-80% homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, even more preferably at least 85-90% homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, and most preferably at least 95% homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11 (e.g., the entire amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11).

An isolated nucleic acid molecule encoding a CSAPTP protein homologous to the protein of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_ by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a CSAPTP protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly

along all or part of a CSAPTP coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for CSAPTP biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:12, 5 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

In a preferred embodiment, a mutant CSAPTP protein can be assayed for the ability to (1) regulate the cell cycle, e.g., dephosphorylation of phosphorylated proteins involved 10 in the cell cycle, e.g., dephosphorylation of phosphorylated proteins involved in the cell cycle, e.g., proliferation-mediating proteins, e.g., Cdc25A, a cell cycle phosphatase which regulates the G1/S-phase transition, e.g., anti-proliferative proteins, either *in vitro*, *in vivo* or *in situ*; (2) mediation of the viral pathogenicity, e.g., viral phosphatase mediated dephosphorylation of host phosphorylated proteins, for example, viral induced 15 disease, e.g., *Yersinia* pathogenesis, for example, *Yersinia pestis* (Bubonic Plague), e.g., viral phosphatase mediated dephosphorylation of host anti-proliferative phosphorylated proteins, for example, viral induced proliferative diseases, e.g., viral induced cancers, either *in vitro*, *in vivo* or *in situ*; (3) regulation of the phosphorylation state of receptors, e.g., insulin receptor, e.g., insulin receptor substrate 1, either *in vitro*, *in vivo* or *in situ*.

20 In addition to the nucleic acid molecules encoding CSAPTP proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or 25 complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire CSAPTP coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding CSAPTP. The term "coding region" 30 includes the region of the nucleotide sequence comprising codons which are translated into amino acid residues (e.g., the coding region of human CSAPTP corresponds to ). In

another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding CSAPTP. The term "noncoding region" includes 5' and 3' sequences which flank the coding region that are not translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions).

5        Given the coding strand sequences encoding CSAPTP disclosed herein (e.g., SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of CSAPTP mRNA, but more preferably is an oligonucleotide which is antisense  
10 to only a portion of the coding or noncoding region of CSAPTP mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of CSAPTP mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and  
15 enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate  
20 derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-  
25 galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine,  
30 uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid

methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a CSAPTP protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An  $\alpha$ -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes  
5 (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave CSAPTP mRNA transcripts to thereby inhibit translation of CSAPTP mRNA. A ribozyme having specificity for a CSAPTP-encoding nucleic acid can be designed based upon the nucleotide sequence of a CSAPTP cDNA disclosed herein (i.e., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7,  
10 SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a CSAPTP-encoding mRNA. See, e.g., Cech *et al.* U.S.  
15 Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, CSAPTP mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, CSAPTP gene expression can be inhibited by targeting nucleotide  
20 sequences complementary to the regulatory region of the CSAPTP (e.g., the CSAPTP promoter and/or enhancers) to form triple helical structures that prevent transcription of the CSAPTP gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

25 In yet another embodiment, the CSAPTP nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal  
30 Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate

backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis  
5 protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* PNAS 93: 14670-675.

PNAs of CSAPTP nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing  
10 transcription or translation arrest or inhibiting replication. PNAs of CSAPTP nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*;  
15 Perry-O'Keefe *supra*).

In another embodiment, PNAs of CSAPTP can be modified, (e.g., to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of CSAPTP  
20 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (e.g., RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of  
25 bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-  
30 thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then

coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

5 In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. US.* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810, published December 15, 1988) or the blood-brain  
10 barrier (see, e.g., PCT Publication No. W089/10134, published April 25, 1988). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol *et al.* (1988) *BioTechniques* 6:958-976) or intercalating agents. (See, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking  
15 agent, transport agent, or hybridization-triggered cleavage agent).

## II. Isolated CSAPTP Proteins and Anti-CSAPTP Antibodies

One aspect of the invention pertains to isolated CSAPTP proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as  
20 immunogens to raise anti-CSAPTP antibodies. In one embodiment, native CSAPTP proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, CSAPTP proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a CSAPTP protein or polypeptide can be synthesized  
25 chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the CSAPTP protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language  
30 "substantially free of cellular material" includes preparations of CSAPTP protein in which the protein is separated from cellular components of the cells from which it is



isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of CSAPTP protein having less than about 30% (by dry weight) of non-CSAPTP protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-CSAPTP protein, 5 still more preferably less than about 10% of non-CSAPTP protein, and most preferably less than about 5% non-CSAPTP protein. When the CSAPTP protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the 10 protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of CSAPTP protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or 15 other chemicals" includes preparations of CSAPTP protein having less than about 30% (by dry weight) of chemical precursors or non-CSAPTP chemicals, more preferably less than about 20% chemical precursors or non-CSAPTP chemicals, still more preferably less than about 10% chemical precursors or non-CSAPTP chemicals, and most preferably less than about 5% chemical precursors or non-CSAPTP chemicals.

20 Biologically active portions of a CSAPTP protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the CSAPTP protein, e.g., the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11, which include less amino acids than the full length CSAPTP proteins, and exhibit at least one activity of a CSAPTP protein. 25 Typically, biologically active portions comprise a domain or motif with at least one activity of the CSAPTP protein. A biologically active portion of a CSAPTP protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

It is to be understood that a preferred biologically active portion of a CSAPTP protein of the present invention may contain at least one of the above-identified 30 structural domains. A more preferred biologically active portion of a CSAPTP protein may contain at least two of the above-identified structural domains. Moreover, other

biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native CSAPTP protein.

In a preferred embodiment, the CSAPTP protein has an amino acid sequence  
5 shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11. In other  
embodiments, the CSAPTP protein is substantially homologous to SEQ ID NO:2, SEQ  
ID NO:5, SEQ ID NO:8, or SEQ ID NO:11 and retains the functional activity of the  
protein of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, yet differs in  
amino acid sequence due to natural allelic variation or mutagenesis, as described in  
10 detail in subsection I above.

Accordingly, in another embodiment, the CSAPTP-1 protein is a protein which  
comprises an amino acid sequence at least 79% homologous to the amino acid sequence  
of SEQ ID NO:2, and retains the functional activity of the CSAPTP-1 proteins of SEQ  
ID NO:2. Preferably, the protein is at least 30-35% homologous to SEQ ID NO:2, more  
15 preferably at least 35-40% homologous to SEQ ID NO:2, even more preferably at least  
40-45% homologous to SEQ ID NO:2, and even more preferably at least 45-50%, 50-  
55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-79%, 79-80%, 80-85%, 85-90%, or 90-  
95% or more homologous to SEQ ID NO:2.

Accordingly, in another embodiment, the CSAPTP-2 protein is a protein which  
20 comprises an amino acid sequence at least 23% homologous to the amino acid sequence  
of SEQ ID NO:5 and retains the functional activity of the CSAPTP-2 proteins of SEQ  
ID NO:5. Preferably, the protein is at least 10%-15% homologous to SEQ ID NO:5,  
more preferably at least 15-20%, more preferably at least 20-23%, more preferably at  
least 23-25%, more preferably at least 25-30%, more preferably at least 30-35%, more  
25 preferably at least 35-40% homologous to SEQ ID NO:5, even more preferably at least  
40-45% homologous to SEQ ID NO:5, and even more preferably at least 45-50%, 50-  
55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-80%, 80-85%, 85-90%, or 90-95% or more  
homologous to SEQ ID NO:5.

Accordingly, in another embodiment, the CSAPTP-3 protein is a protein which  
30 comprises an amino acid sequence at least 24% homologous to the amino acid sequence  
of SEQ ID NO:8 and retains the functional activity of the CSAPTP-3 proteins of SEQ

ID NO:8. Preferably, the protein is at least 10-15%, 15-20%, 20-24%, 24-25%, 25-30%, 30-35% homologous to SEQ ID NO:8, more preferably at least 35-40% homologous to SEQ ID NO:8, even more preferably at least 40-45% homologous to SEQ ID NO:8, and even more preferably at least 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-80%, 80-85%, 85-90%, or 90-95% or more homologous to SEQ ID NO:8.

Accordingly, in another embodiment, the CSAPTP-4 protein is a protein which comprises an amino acid sequence at least about 42% or more homologous to the amino acid sequence of SEQ ID NO:11, and retains the functional activity of the CSAPTP-4 proteins of SEQ ID NO:11. Preferably, the protein is at least about 10-15% to SEQ ID NO:11, more preferably at least about 15-20% to SEQ ID NO:11, more preferably at least about 20-25% to SEQ ID NO:11, more preferably at least about 25-30% to SEQ ID NO:11, more preferably at least about 30-35% homologous to SEQ ID NO:11, more preferably at least about 35-40% homologous to SEQ ID NO:11, even more preferably at least about 40-42% homologous to SEQ ID NO:11, and even more preferably at least about 42-45%, 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-80%, 80-85%, 85-90%, or 90-95% or more homologous to SEQ ID NO:11.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (e.g., when aligning a second sequence to the CSAPTP amino acid sequence of SEQ ID NO:2 having 173 amino acid residues, at least 52, preferably at least 70, more preferably at least 87, even more preferably at least 104, and even more preferably at least 121, 138 or 158 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that

position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to CSAPTP nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to CSAPTP protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25(17):3389-3402.

When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

The invention also provides CSAPTP chimeric or fusion proteins. As used herein, a CSAPTP "chimeric protein" or "fusion protein" comprises a CSAPTP polypeptide operatively linked to a non-CSAPTP polypeptide. A "CSAPTP polypeptide" includes a polypeptide having an amino acid sequence corresponding to CSAPTP, whereas a "non-CSAPTP polypeptide" includes a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the CSAPTP protein, e.g., a protein which is different from the CSAPTP protein and which is derived from the same or a different organism. Within a CSAPTP fusion protein the CSAPTP polypeptide can correspond to all or a portion of a CSAPTP protein. In a preferred embodiment, a CSAPTP fusion protein comprises at least one biologically active portion of a CSAPTP protein. In another preferred embodiment, a CSAPTP fusion protein comprises at least two biologically active portions of a CSAPTP protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the CSAPTP polypeptide and the non-CSAPTP polypeptide are fused in-frame to each other. The non-CSAPTP polypeptide can be fused to the N-terminus or C-terminus of the CSAPTP polypeptide.

For example, in one embodiment, the fusion protein is a GST-CSAPTP fusion protein in which the CSAPTP sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant CSAPTP.

In another embodiment, the fusion protein is a CSAPTP protein containing a heterologous signal sequence at its N-terminus. For example, the native CSAPTP signal sequence can be removed and replaced with a signal sequence from another protein. In certain host cells (e.g., mammalian host cells), expression and/or secretion of CSAPTP can be increased through use of a heterologous signal sequence.

The CSAPTP fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The CSAPTP fusion proteins can be used to affect the bioavailability of a CSAPTP target molecule. Use of

CSAPTP fusion proteins may be useful therapeutically for the treatment of proliferative disorders (e.g., cancer). Moreover, the CSAPTP-fusion proteins of the invention can be used as immunogens to produce anti-CSAPTP antibodies in a subject, to purify CSAPTP ligands and in screening assays to identify molecules which inhibit the  
5 interaction of CSAPTP with a CSAPTP target molecule.

Preferably, a CSAPTP chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended  
10 termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor  
15 primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A CSAPTP-  
20 encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the CSAPTP protein.

The present invention also pertains to variants of the CSAPTP proteins which function as either CSAPTP agonists (mimetics) or as CSAPTP antagonists. Variants of the CSAPTP proteins can be generated by mutagenesis, e.g., discrete point mutation or  
25 truncation of a CSAPTP protein. An agonist of the CSAPTP proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a CSAPTP protein. An antagonist of a CSAPTP protein can inhibit one or more of the activities of the naturally occurring form of the CSAPTP protein by, for example, competitively inhibiting the phosphatase activity of a CSAPTP protein. Thus, specific  
30 biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological

activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the CSAPTP protein.

In one embodiment, variants of a CSAPTP protein which function as either CSAPTP agonists (mimetics) or as CSAPTP antagonists can be identified by screening  
5 combinatorial libraries of mutants, e.g., truncation mutants, of a CSAPTP protein for CSAPTP protein agonist or antagonist activity. In one embodiment, a variegated library of CSAPTP variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of CSAPTP variants can be produced by, for example, enzymatically ligating a mixture of synthetic  
10 oligonucleotides into gene sequences such that a degenerate set of potential CSAPTP sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of CSAPTP sequences therein. There are a variety of methods which can be used to produce libraries of potential CSAPTP variants from a degenerate oligonucleotide sequence. Chemical  
15 synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential CSAPTP sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang, S.A.  
20 (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of a CSAPTP protein coding sequence can be used to generate a variegated population of CSAPTP fragments for screening and subsequent selection of variants of a CSAPTP protein. In one embodiment, a library of  
25 coding sequence fragments can be generated by treating a double stranded PCR fragment of a CSAPTP coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed  
30 duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into

an expression vector. By this method, an expression library can be derived which encodes N-terminal, and internal fragments of various sizes of the CSAPTP protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA  
5 libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of CSAPTP proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the  
10 resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify CSAPTP variants (Arkin and Yourvan  
15 (1992) *PNAS* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

In one embodiment, cell based assays can be exploited to analyze a variegated CSAPTP library. For example, a library of expression vectors can be transfected into a cell line which ordinarily synthesizes and secretes CSAPTP. The transfected cells are then cultured such that CSAPTP and a particular mutant CSAPTP are secreted and the  
20 effect of expression of the mutant on CSAPTP activity in cell supernatants can be detected, e.g., by any of a number of enzymatic assays. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of CSAPTP activity, and the individual clones further characterized.

An isolated CSAPTP protein, or a portion or fragment thereof, can be used as an  
25 immunogen to generate antibodies that bind CSAPTP using standard techniques for polyclonal and monoclonal antibody preparation. A full-length CSAPTP protein can be used or, alternatively, the invention provides antigenic peptide fragments of CSAPTP for use as immunogens. The antigenic peptide of a CSAPTP protein comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5,  
30 SEQ ID NO:8, or SEQ ID NO:11 and encompasses an epitope of CSAPTP such that an antibody raised against the peptide forms a specific immune complex with CSAPTP.



Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of CSAPTP  
5 that are located on the surface of the protein, e.g., hydrophilic regions.

A CSAPTP immunogen typically is used to prepare antibodies by immunizing a suitable subject, (e.g., rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed CSAPTP protein or a chemically synthesized CSAPTP polypeptide. The  
10 preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic CSAPTP preparation induces a polyclonal anti-CSAPTP antibody response.

Accordingly, another aspect of the invention pertains to anti-CSAPTP  
15 antibodies. The term "antibody" as used herein includes immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as CSAPTP. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')<sub>2</sub> fragments which can be generated by treating the  
20 antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind CSAPTP. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, includes a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of CSAPTP. A monoclonal antibody  
25 composition thus typically displays a single binding affinity for a particular CSAPTP protein with which it immunoreacts.

Polyclonal anti-CSAPTP antibodies can be prepared as described above by immunizing a suitable subject with a CSAPTP immunogen. The anti-CSAPTP antibody titer in the immunized subject can be monitored over time by standard techniques, such  
30 as with an enzyme linked immunosorbent assay (ELISA) using immobilized CSAPTP. If desired, the antibody molecules directed against CSAPTP can be isolated from the

mammal (e.g., from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-CSAPTP antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal

5 antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *PNAS* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72),

10 the EBV-hybridoma technique (Cole *et al.* (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*,

15 54:387-402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a CSAPTP immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds CSAPTP.

20 Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-CSAPTP monoclonal antibody (see, e.g., G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will

25 appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell

30 lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of

- myeloma cell lines can be used as a fusion partner according to standard techniques, e.g., the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind CSAPTP, e.g., using a standard ELISA assay.
- 10           Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-CSAPTP antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with CSAPTP to thereby isolate immunoglobulin library members that bind CSAPTP. Kits for generating and screening phage display libraries are commercially
- 15           available (e.g., the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO
- 20           92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International
- 25           Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrard *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.*
- 30           (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *PNAS* 88:7978-7982; and McCafferty *et al.* *Nature* (1990) 348:552-554.

Additionally, recombinant anti-CSAPTP antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by

5 recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567;

10 Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *PNAS* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *PNAS* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.*

15 (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

An anti-CSAPTP antibody (e.g., monoclonal antibody) can be used to isolate CSAPTP by standard techniques, such as affinity chromatography or

20 immunoprecipitation. An anti-CSAPTP antibody can facilitate the purification of natural CSAPTP from cells and of recombinantly produced CSAPTP expressed in host cells. Moreover, an anti-CSAPTP antibody can be used to detect CSAPTP protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the CSAPTP protein. Anti-CSAPTP antibodies can be used diagnostically

25 to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive

30 materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic

group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

### III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a CSAPTP protein (or a portion thereof). As used herein, the term "vector" includes a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which includes a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is

operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., CSAPTP proteins, mutant forms of CSAPTP proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of CSAPTP proteins in prokaryotic or eukaryotic cells. For example, CSAPTP proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein;

2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in CSAPTP activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for CSAPTP proteins, for example. In a preferred embodiment, a CSAPTP fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six (6) weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another

strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard  
5 DNA synthesis techniques.

In another embodiment, the CSAPTP expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen  
10 Corporation, San Diego, CA), and picZ (InVitrogen Corp, San Diego, CA).

Alternatively, CSAPTP proteins can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology*  
15 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the  
20 expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed.*, Cold Spring  
25 Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-  
30 specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.*



(1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters  
5 (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990)  
10 *Science* 249:374-379) and the  $\alpha$ -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in  
15 a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to CSAPTP mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be  
20 chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the  
25 regulation of gene expression using antisense genes see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and  
30 "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny

of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

5           A host cell can be any prokaryotic or eukaryotic cell. For example, a CSAPTP protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

          Vector DNA can be introduced into prokaryotic or eukaryotic cells via  
10   conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or  
15   transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual*, 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

          For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may  
20   integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be  
25   introduced into a host cell on the same vector as that encoding a CSAPTP protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

          A host cell of the invention, such as a prokaryotic or eukaryotic host cell in  
30   culture, can be used to produce (i.e., express) a CSAPTP protein. Accordingly, the invention further provides methods for producing a CSAPTP protein using the host cells

of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding a CSAPTP protein has been introduced) in a suitable medium such that a CSAPTP protein is produced. In another embodiment, the method further comprises isolating a CSAPTP protein from the  
5 medium or the host cell.

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which CSAPTP-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in  
10 which exogenous CSAPTP sequences have been introduced into their genome or homologous recombinant animals in which endogenous CSAPTP sequences have been altered. Such animals are useful for studying the function and/or activity of a CSAPTP and for identifying and/or evaluating modulators of CSAPTP activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a  
15 rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an  
20 encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous CSAPTP gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the  
25 animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing a CSAPTP-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The CSAPTP cDNA sequence of SEQ ID NO:1,  
30 SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:12 can be introduced as a transgene into the genome of a non-

human animal. Alternatively, a nonhuman homologue of a human CSAPTP gene, such as a mouse or rat CSAPTP gene, can be used as a transgene. Alternatively, a CSAPTP gene homologue, such as another CSAPTP family member, can be isolated based on hybridization to the CSAPTP cDNA sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_ (described further in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a CSAPTP transgene to direct expression of a CSAPTP protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a CSAPTP transgene in its genome and/or expression of CSAPTP mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a CSAPTP protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a CSAPTP gene into which a deletion, addition or substitution has been introduced to thereby alter, e.g., functionally disrupt, the CSAPTP gene. The CSAPTP gene can be a human gene (e.g., the cDNA of ), but more preferably, is a non-human homologue of a human CSAPTP gene (e.g., a cDNA isolated by stringent hybridization with the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:12). For example, a mouse CSAPTP gene can be used to construct a homologous recombination vector suitable for altering an endogenous CSAPTP gene in the mouse

genome. In a preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous CSAPTP gene is functionally disrupted (i.e., no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous

5 CSAPTP gene is mutated or otherwise altered but still encodes functional protein (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous CSAPTP protein). In the homologous recombination vector, the altered portion of the CSAPTP gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the CSAPTP gene to allow for homologous recombination to occur between

10 the exogenous CSAPTP gene carried by the vector and an endogenous CSAPTP gene in an embryonic stem cell. The additional flanking CSAPTP nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (see e.g., Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a

15 description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced CSAPTP gene has homologously recombined with the endogenous CSAPTP gene are selected (see e.g., Li, E. *et al.* (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (see e.g.,

20 Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously

25 recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

In another embodiment, transgenic non-humans animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, e.g., Lakso *et al.* (1992) *PNAS* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813. In brief, a cell, e.g., a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G<sub>0</sub> phase. Alternatively, a cell, e.g., an embryonic stem cell, from the inner cell mass of a developing embryo can be transformed with a preferred transgene. Alternatively, a cell, e.g., a somatic cell, from cell culture line can be transformed with a preferred transgene and induced to exit the growth cycle and enter G<sub>0</sub> phase. The cell can then be fused, e.g., through the use of electrical pulses, to an enucleated mammalian oocyte. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the nuclear donor cell, e.g., the somatic cell, is isolated.

25

#### IV. Pharmaceutical Compositions

The CSAPTP nucleic acid molecules, CSAPTP proteins, and anti-CSAPTP antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a

30

pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as  
5 any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible  
10 with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils,  
15 polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases,  
20 such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the  
25 extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage  
30 and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for

example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of  
5 surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be  
10 brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a CSAPTP protein or anti-CSAPTP antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as  
15 required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a  
20 powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and  
25 used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the  
30 following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or



lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

- 5           For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

          Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be  
10 permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in  
15 the art.

          The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

- In one embodiment, the active compounds are prepared with carriers that will  
20 protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art.
- 25           The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein includes physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound

5 calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

10 Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio  
15 LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in  
20 formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially  
25 from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high  
30 performance liquid chromatography.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen *et al.* (1994) *PNAS* 91:3054-3057). The  
5 pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the  
10 pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

#### V. Uses and Methods of the Invention

15 The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (e.g., therapeutic and prophylactic).

20 As described herein, a CSAPTP protein of the invention has one or more of the following activities: (i) interaction of a CSAPTP protein with a CSAPTP target molecule; (ii) interaction of a CSAPTP protein with a CSAPTP target molecule, wherein the CSAPTP target is a ligand, e.g., phosphorylated amino acid residue of a phosphorylated protein, e.g., a phosphatase, for example, a cell cycle regulatory  
25 phosphatase, e.g., Cdc25A phosphatase, a cell cycle phosphatase which regulates the G1/S-phase transition, a CSAPTPase, a kinase, e.g., Mitogen Activating Protein (MAP) kinase, or a Cardiovascular Associated Protein Tyrosine Kinase (CSAPTK); (iii) interaction of a CSAPTP protein with a CSAPTP target molecule, wherein the CSAPTP target is a receptor, e.g., insulin receptor, insulin receptor substrate 1; (iv) interaction of  
30 a CSAPTP protein with a CSAPTP target molecule, wherein the CSAPTP target is a viral protein, e.g., *vaccinia* viral transcription-mediating proteins, Myxoma viral

proteins, Shope Fibroma viral proteins, *Leishmania donovani*, *Trypanosoma brucei* and *Trypanosoma cruzi* viral proteins.

Further, as described herein, a CSAPTP protein of the invention has one or more of the above activities and can thus be used in, for example, the: (1) regulation of cell cycle, e.g., dephosphorylation of phosphorylated proteins involved in the cell cycle, e.g., proliferation-mediating proteins, e.g., Cdc25A, a cell cycle phosphatase which regulates the G1/S-phase transition, e.g., anti-proliferative proteins, either *in vitro*, *in vivo* or *in situ*; (2) mediation of viral pathogenicity, e.g., viral phosphatase mediated dephosphorylation of host phosphorylated proteins, for example, viral induced disease, e.g., *Yersinia* pathogenesis, for example, *Yersinia pestis* (Bubonic Plague), e.g., viral phosphatase mediated dephosphorylation of host anti-proliferative phosphorylated proteins, for example, viral induced proliferative diseases, e.g., viral induced cancers, either *in vitro*, *in vivo* or *in situ*; (3) regulation of the phosphorylation state of receptors, e.g., insulin receptor, e.g., insulin receptor substrate 1, either *in vitro*, *in vivo* or *in situ*.

The isolated nucleic acid molecules of the invention can be used, for example, to express CSAPTP protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect CSAPTP mRNA (e.g., in a biological sample) or a genetic alteration in a CSAPTP gene, and to modulate CSAPTP activity, as described further below. The CSAPTP proteins can be used to treat disorders characterized by insufficient or excessive production of a CSAPTP or CSAPTP target molecules. In addition, the CSAPTP proteins can be used to screen for naturally occurring CSAPTP target molecules, to screen for drugs or compounds which modulate CSAPTP activity, as well as to treat disorders characterized by insufficient or excessive production of CSAPTP protein or production of CSAPTP protein forms which have decreased or aberrant activity compared to CSAPTP wild type protein. Moreover, the anti-CSAPTP antibodies of the invention can be used to detect and isolate CSAPTP proteins, regulate the bioavailability of CSAPTP proteins, and modulate CSAPTP activity.

Accordingly one embodiment of the present invention involves a method of use (e.g., a diagnostic assay, prognostic assay, or a prophylactic/therapeutic method of treatment) wherein a molecule of the present invention (e.g., a CSAPTP protein, CSAPTP nucleic acid, or a CSAPTP modulator) is used, for example, to diagnose,

prognose and/or treat a disease and/or condition in which any of the aforementioned activities (i.e., activities (i) - (iv) and (1) - (3) in the above paragraph) is indicated. In another embodiment, the present invention involves a method of use (e.g., a diagnostic assay, prognostic assay, or a prophylactic/therapeutic method of treatment) wherein a molecule of the present invention (e.g., a CSAPTP protein, CSAPTP nucleic acid, or a CSAPTP modulator) is used, for example, for the diagnosis, prognosis, and/or treatment of subjects, preferably a human subject, in which any of the aforementioned activities is pathologically perturbed. In a preferred embodiment, the methods of use (e.g., diagnostic assays, prognostic assays, or prophylactic/therapeutic methods of treatment) involve administering to a subject, preferably a human subject, a molecule of the present invention (e.g., a CSAPTP protein, CSAPTP nucleic acid, or a CSAPTP modulator) for the diagnosis, prognosis, and/or therapeutic treatment. In another embodiment, the methods of use (e.g., diagnostic assays, prognostic assays, or prophylactic/therapeutic methods of treatment) involve administering to a human subject a molecule of the present invention (e.g., a CSAPTP protein, CSAPTP nucleic acid, or a CSAPTP modulator).

A. Screening Assays:

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other drugs) which bind to CSAPTP proteins, have a stimulatory or inhibitory effect on, for example, CSAPTP expression or CSAPTP activity, or have a stimulatory or inhibitory effect on, for example, the activity of an CSAPTP target molecule.

In one embodiment, the invention provides assays for screening candidate or test compounds which are target molecules of a CSAPTP protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a CSAPTP protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological

libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a CSAPTP protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate CSAPTP activity determined. Determining the ability of the test compound to modulate CSAPTP activity can be accomplished by monitoring the bioactivity of the CSAPTP protein or biologically active portion thereof. The cell, for example, can be of mammalian origin or a yeast cell. Determining the ability of the test compound to modulate CSAPTP activity can be accomplished, for example, by coupling the CSAPTP protein or biologically active portion thereof with a radioisotope or enzymatic label such that binding of the CSAPTP protein or biologically active portion thereof to its cognate target molecule can be determined by detecting the labeled CSAPTP protein or biologically active portion thereof in a complex. For example, compounds (e.g.,

CSAPTP protein or biologically active portion thereof) can be labeled with  $^{125}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ , or  $^3\text{H}$ , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (e.g., CSAPTP protein or biologically active portion thereof) to interact with its cognate target molecule without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with its cognate target molecule without the labeling of either the compound or the receptor. McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (e.g., Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between compound and receptor.

In a preferred embodiment, the assay comprises contacting a cell which expresses a CSAPTP protein or biologically active portion thereof, with a target molecule to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to modulate the activity of the CSAPTP protein or biologically active portion thereof, wherein determining the ability of the test compound to modulate the activity of the CSAPTP protein or biologically active portion thereof, comprises determining the ability of the test compound to modulate a biological activity of the CSAPTP expressing cell (e.g., determining the ability of the test compound to modulate cell proliferation, viral replication, and/or receptor regulation).

In another preferred embodiment, the assay comprises contacting a cell which is responsive to a CSAPTP protein or biologically active portion thereof, with a CSAPTP protein or biologically-active portion thereof, to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to modulate the activity of the CSAPTP protein or biologically active portion thereof,

wherein determining the ability of the test compound to modulate the activity of the CSAPTP protein or biologically active portion thereof comprises determining the ability of the test compound to modulate a biological activity of the CSAPTP-responsive cell (e.g., determining the ability of the test compound to modulate cell proliferation, viral replication, and/or receptor regulation).

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a CSAPTP target molecule with a test compound and determining the ability of the test compound to modulate (e.g. stimulate or inhibit) the activity of the CSAPTP target molecule. Determining the ability of the test compound to modulate the activity of a CSAPTP target molecule can be accomplished, for example, by determining the ability of the CSAPTP protein to bind to or interact with the CSAPTP target molecule.

Determining the ability of the CSAPTP protein to bind to or interact with a CSAPTP target molecule can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the CSAPTP protein to bind to or interact with a CSAPTP target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting dephosphorylation of a phosphorylated protein. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (i.e. intracellular  $\text{Ca}^{2+}$ , diacylglycerol,  $\text{IP}_3$ , etc.), detecting catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, e.g., luciferase), or detecting a target-regulated cellular response, for example, cell proliferation, viral replication, and/or receptor regulation.

In yet another embodiment, an assay of the present invention is a cell-free assay in which a CSAPTP protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the CSAPTP protein or biologically active portion thereof is determined. Binding of the test compound to the CSAPTP protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the CSAPTP protein or



biologically active portion thereof with a known compound which binds CSAPTP (e.g., a CSAPTP target molecule) to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a CSAPTP protein, wherein determining the ability of the test compound to interact with a

5 CSAPTP protein comprises determining the ability of the test compound to preferentially bind to CSAPTP or biologically active portion thereof as compared to the known compound.

In another embodiment, the assay is a cell-free assay in which a CSAPTP protein or biologically active portion thereof is contacted with a test compound and the ability of

10 the test compound to modulate (e.g., stimulate or inhibit) the activity of the CSAPTP protein or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a CSAPTP protein can be accomplished, for example, by determining the ability of the CSAPTP protein to bind to a CSAPTP target molecule by one of the methods described above for determining direct binding.

15 Determining the ability of the CSAPTP protein to bind to a CSAPTP target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the

20 interactants (e.g., BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of a CSAPTP protein can be accomplished by determining the

25 ability of the CSAPTP protein to further modulate the activity of a downstream effector (e.g., a transcriptionally activated immediate early response pathway component) of a CSAPTP target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting a CSAPTP protein or biologically active portion thereof with a known compound which binds the CSAPTP protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the CSAPTP protein, wherein determining the ability of the test compound to interact with the CSAPTP protein comprises determining the ability of the CSAPTP protein to preferentially bind to or modulate the activity of a CSAPTP target molecule.

The cell-free assays of the present invention are amenable to use of both soluble and/or membrane-bound forms of isolated proteins (e.g. CSAPTP proteins or biologically active portions thereof or receptors to which CSAPTP targets bind). In the case of cell-free assays in which a membrane-bound form of an isolated protein is used (e.g., a cell surface receptor) it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the isolated protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)<sub>n</sub>, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either CSAPTP or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a CSAPTP protein, or interaction of a CSAPTP protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/ CSAPTP fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre

plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or CSAPTP protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove  
5 any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of CSAPTP binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the  
10 screening assays of the invention. For example, either a CSAPTP protein or a CSAPTP target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated CSAPTP protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96  
15 well plates (Pierce Chemical). Alternatively, antibodies reactive with CSAPTP protein or target molecules but which do not interfere with binding of the CSAPTP protein to its target molecule can be derivatized to the wells of the plate, and unbound target or CSAPTP protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized  
20 complexes, include immunodetection of complexes using antibodies reactive with the CSAPTP protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the CSAPTP protein or target molecule.

In another embodiment, modulators of CSAPTP expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of  
25 CSAPTP mRNA or protein in the cell is determined. The level of expression of CSAPTP mRNA or protein in the presence of the candidate compound is compared to the level of expression of CSAPTP mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of CSAPTP expression based on this comparison. For example, when expression of CSAPTP  
30 mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a

stimulator of CSAPTP mRNA or protein expression. Alternatively, when expression of CSAPTP mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of CSAPTP mRNA or protein expression. The level of CSAPTP mRNA or protein expression in the cells can be determined by methods described herein for  
5 detecting CSAPTP mRNA or protein.

In yet another aspect of the invention, the CSAPTP proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.*  
10 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with CSAPTP ("CSAPTP-binding proteins" or "CSAPTP-bp") and are involved in CSAPTP activity. Such CSAPTP-binding proteins are also likely to be involved in the propagation of signals by the CSAPTP proteins or CSAPTP targets  
15 as, for example, downstream elements of a CSAPTP-mediated signaling pathway. Alternatively, such CSAPTP-binding proteins are likely to be CSAPTP inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a  
20 CSAPTP protein is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a CSAPTP-  
25 dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the  
30 cloned gene which encodes the protein which interacts with the CSAPTP protein.

This invention further pertains to novel agents identified by the above-described screening assays and to processes for producing such agents by use of these assays. Accordingly, in one embodiment, the present invention includes a compound or agent obtainable by a method comprising the steps of any one of the aforementioned screening  
5 assays (e.g., cell-based assays or cell-free assays). For example, in one embodiment, the invention includes a compound or agent obtainable by a method comprising contacting a cell which expresses a CSAPTP target molecule with a test compound and the determining the ability of the test compound to bind to, or modulate the activity of, the CSAPTP target molecule. In another embodiment, the invention includes a compound  
10 or agent obtainable by a method comprising contacting a cell which expresses a CSAPTP target molecule with a CSAPTP protein or biologically-active portion thereof, to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with, or modulate the activity of, the CSAPTP target molecule. In another embodiment, the invention includes a  
15 compound or agent obtainable by a method comprising contacting a CSAPTP protein or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to, or modulate (e.g., stimulate or inhibit) the activity of, the CSAPTP protein or biologically active portion thereof. In yet another embodiment, the present invention included a compound or agent obtainable by a method comprising  
20 contacting a CSAPTP protein or biologically active portion thereof with a known compound which binds the CSAPTP protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with, or modulate the activity of the CSAPTP protein.

Accordingly, it is within the scope of this invention to further use an agent  
25 identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a CSAPTP modulating agent, an antisense CSAPTP nucleic acid molecule, a CSAPTP-specific antibody, or a CSAPTP-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can  
30 be used in an animal model to determine the mechanism of action of such an agent.

Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

The present invention also pertains to uses of novel agents identified by the above-described screening assays for diagnoses, prognoses, and treatments as described  
5 herein. Accordingly, it is within the scope of the present invention to use such agents in the design, formulation, synthesis, manufacture, and/or production of a drug or pharmaceutical composition for use in diagnosis, prognosis, or treatment, as described herein. For example, in one embodiment, the present invention includes a method of synthesizing or producing a drug or pharmaceutical composition by reference to the  
10 structure and/or properties of a compound obtainable by one of the above-described screening assays. For example, a drug or pharmaceutical composition can be synthesized based on the structure and/or properties of a compound obtained by a method in which a cell which expresses a CSAPTP target molecule is contacted with a test compound and the ability of the test compound to bind to, or modulate the activity  
15 of, the CSAPTP target molecule is determined. In another exemplary embodiment, the present invention includes a method of synthesizing or producing a drug or pharmaceutical composition based on the structure and/or properties of a compound obtainable by a method in which a CSAPTP protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind  
20 to, or modulate (e.g., stimulate or inhibit) the activity of, the CSAPTP protein or biologically active portion thereof is determined.

#### B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the  
25 corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications  
30 are described in the subsections below.

### 1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the CSAPTP nucleotide sequences, described herein, can be used to map the location of the CSAPTP genes on a chromosome. The mapping of the CSAPTP sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, CSAPTP genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the CSAPTP nucleotide sequences.

Computer analysis of the CSAPTP sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the CSAPTP sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the CSAPTP nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can

similarly be used to map a 9o, 1p, or 1v sequence to its chromosome include *in situ* hybridization (described in Fan, Y. *et al.* (1990) *PNAS*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

5           Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A  
10       pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will  
15       suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

          Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for  
20       marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

          Once a sequence has been mapped to a precise chromosomal location, the  
25       physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes),  
30       described in, for example, Egeland, J. *et al.* (1987) *Nature*, 325:783-787.



Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the CSAPTP gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease.

- 5 Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

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## 2. Tissue Typing

- The CSAPTP sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for
- 15 identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers
- 20 for RFLP (described in U.S. Patent 5,272,057).

- Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the CSAPTP nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the
- 25 sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

- Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the
- 30 present invention can be used to obtain such identification sequences from individuals and from tissue. The CSAPTP nucleotide sequences of the invention uniquely represent

portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:12 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:12 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from CSAPTP nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

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### 3. Use of Partial CSAPTP Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:12 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the CSAPTP nucleotide sequences or portions thereof, e.g., fragments derived from the noncoding regions of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:12 having a length of at least 20 bases, preferably at least 30 bases.

The CSAPTP nucleotide sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, e.g., brain tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such CSAPTP probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, e.g., CSAPTP primers or probes can be used to screen tissue culture for contamination (i.e. screen for the presence of a mixture of different types of cells in a culture).

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### C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining CSAPTP protein and/or nucleic acid expression as well as CSAPTP

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activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant CSAPTP expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with CSAPTP protein, nucleic acid expression or activity. For example, mutations in a CSAPTP gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with CSAPTP protein, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of CSAPTP in clinical trials.

These and other agents are described in further detail in the following sections.

#### 1. Diagnostic Assays

An exemplary method for detecting the presence or absence of CSAPTP protein or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting CSAPTP protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes CSAPTP protein such that the presence of CSAPTP protein or nucleic acid is detected in the biological sample. A preferred agent for detecting CSAPTP mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to CSAPTP mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length CSAPTP nucleic acid, such as the nucleic acid of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 or SEQ ID NO:10 (or that of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12, or the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, or a portion thereof), such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to CSAPTP mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting CSAPTP protein is an antibody capable of binding to CSAPTP protein, preferably an antibody with a detectable label. Antibodies

can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect CSAPTP mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of CSAPTP mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of CSAPTP protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of CSAPTP genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of CSAPTP protein include introducing into a subject a labeled anti-CSAPTP antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting CSAPTP protein, mRNA, or genomic DNA, such that the presence of CSAPTP protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of CSAPTP protein, mRNA or genomic DNA in the control sample with the presence of CSAPTP protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of CSAPTP in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting CSAPTP protein or mRNA in a biological sample; means for determining the amount of CSAPTP in the sample; and means for comparing the amount of CSAPTP in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect CSAPTP protein or nucleic acid.

## 2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant CSAPTP expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with CSAPTP protein, nucleic acid expression or activity such a proliferative disorder (e.g., cancer). Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a metabolic disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant CSAPTP expression or activity in which a test sample is obtained from a subject and CSAPTP protein or nucleic acid (e.g., mRNA, genomic DNA) is detected, wherein the presence of CSAPTP protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant CSAPTP expression or activity. As used herein, a "test sample" includes a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant CSAPTP expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a proliferative disorder (e.g., cancer). For example, such methods can be used to determine whether a subject can be effectively treated with

an agent for a metabolic disorder. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant CSAPTP expression or activity in which a test sample is obtained and CSAPTP protein or nucleic acid expression or activity is detected (e.g.,  
5 wherein the abundance of CSAPTP protein or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant CSAPTP expression or activity.)

The methods of the invention can also be used to detect genetic alterations in a CSAPTP gene, thereby determining if a subject with the altered gene is at risk for a  
10 disorder characterized by aberrant developmental progression. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a CSAPTP-protein, or the mis-expression of the CSAPTP gene. For example, such genetic alterations can be detected by  
15 ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a CSAPTP gene; 2) an addition of one or more nucleotides to a CSAPTP gene; 3) a substitution of one or more nucleotides of a CSAPTP gene, 4) a chromosomal rearrangement of a CSAPTP gene; 5) an alteration in the level of a messenger RNA transcript of a CSAPTP gene, 6) aberrant modification of a CSAPTP gene, such as of  
20 the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a CSAPTP gene, 8) a non-wild type level of a CSAPTP-protein, 9) allelic loss of a CSAPTP gene, and 10) inappropriate post-translational modification of a CSAPTP-protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting alterations  
25 in a CSAPTP gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a  
30 ligation chain reaction (LCR) (see, e.g., Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *PNAS* 91:360-364), the latter of which can be particularly

useful for detecting point mutations in the CSAPTP-gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more  
5 primers which specifically hybridize to a CSAPTP gene under conditions such that hybridization and amplification of the CSAPTP-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in  
10 conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, 1990, *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.*, 1989, *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.*, 1988, *Bio/Technology* 6:1197), or any  
15 other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a CSAPTP gene from a sample cell  
20 can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence  
25 specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in CSAPTP can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, to high density  
30 arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-



759). For example, genetic mutations in CSAPTP can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the CSAPTP gene and detect mutations by comparing the sequence of the sample CSAPTP with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert ((1977) *PNAS* 74:560) or Sanger ((1977) *PNAS* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, e.g., PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the CSAPTP gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type CSAPTP sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with

hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* 5 (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point 10 mutations in CSAPTP cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a CSAPTP sequence, e.g., a wild-type CSAPTP sequence, is hybridized to a cDNA or other DNA 15 product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in CSAPTP genes. For example, single strand conformation 20 polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat Res* 285:125-144; and Hayashi (1992) *Genet Anal Tech Appl* 9:73-79). Single-stranded DNA fragments of sample and control CSAPTP nucleic acids will be denatured and allowed to renature. The secondary 25 structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the 30 subject method utilizes heteroduplex analysis to separate double stranded heteroduplex

molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing  
5 gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control  
10 and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions  
15 which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

20 Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme  
25 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for  
30 amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it

possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a CSAPTP gene.

Furthermore, any cell type or tissue in which CSAPTP is expressed may be utilized in the prognostic assays described herein.

10

### 3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of a CSAPTP protein (*e.g.*, modulation of cell proliferation, *e.g.*, dephosphorylation of phosphorylated cell cycle proteins) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase CSAPTP gene expression, protein levels, or upregulate CSAPTP activity, can be monitored in clinical trials of subjects exhibiting decreased CSAPTP gene expression, protein levels, or downregulated CSAPTP activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease CSAPTP gene expression, protein levels, or downregulate CSAPTP activity, can be monitored in clinical trials of subjects exhibiting increased CSAPTP gene expression, protein levels, or upregulated CSAPTP activity. In such clinical trials, the expression or activity of a CSAPTP gene, and preferably, other genes that have been implicated in, for example, a developmental disorder can be used as a "read out" or markers of the phenotype of a particular cell.

For example, and not by way of limitation, genes, including CSAPTP, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates CSAPTP activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on proliferative disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of CSAPTP and other genes implicated in a proliferative,

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differentiative, or metabolic disorder. The levels of gene expression (i.e., a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of CSAPTP or other  
5 genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for  
10 monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a CSAPTP protein, mRNA, or genomic  
15 DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the CSAPTP protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the CSAPTP protein, mRNA, or genomic DNA in the pre-administration sample with the CSAPTP protein, mRNA, or  
20 genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of CSAPTP to higher levels than detected, i.e., to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease  
25 expression or activity of CSAPTP to lower levels than detected, i.e. to decrease the effectiveness of the agent. According to such an embodiment, CSAPTP expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

C. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant CSAPTP expression or activity. With regards to both prophylactic and  
5 therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, includes the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of  
10 how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the CSAPTP molecules of the present invention or CSAPTP modulators according to that individual's drug response genotype. Pharmacogenomics  
15 allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

1. Prophylactic Methods

20 In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant CSAPTP expression or activity, by administering to the subject a CSAPTP or an agent which modulates CSAPTP expression or at least one CSAPTP activity. Subjects at risk for a disease which is caused or contributed to by aberrant CSAPTP expression or activity can be identified by,  
25 for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the CSAPTP aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of CSAPTP aberrancy, for example, a CSAPTP, CSAPTP agonist or CSAPTP antagonist  
30 agent can be used for treating the subject. The appropriate agent can be determined

based on screening assays described herein. The prophylactic methods of the present invention are further discussed in the following subsections.

## 2. Therapeutic Methods

5 Another aspect of the invention pertains to methods of modulating CSAPTP expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a CSAPTP or agent that modulates one or more of the activities of CSAPTP protein activity associated with the cell. An agent that modulates CSAPTP protein activity can  
10 be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a CSAPTP protein, a CSAPTP antibody, a CSAPTP agonist or antagonist, a peptidomimetic of a CSAPTP agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more CSAPTP activities. Examples of such stimulatory agents include active CSAPTP protein and a nucleic acid  
15 molecule encoding CSAPTP that has been introduced into the cell. In another embodiment, the agent inhibits one or more CSAPTP activities. Examples of such inhibitory agents include antisense CSAPTP nucleic acid molecules, anti-CSAPTP antibodies, and CSAPTP inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent), *in vivo* (e.g., by administering the agent  
20 to a subject), or alternatively *in situ* (e.g., at the site of lesion or injury, for example, in the hematopoietic system, e.g., bone marrow). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a CSAPTP protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a  
25 screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) CSAPTP expression or activity. In another embodiment, the method involves administering a CSAPTP protein or nucleic acid molecule as therapy to compensate for reduced or aberrant CSAPTP expression or activity.

Stimulation of CSAPTP activity is desirable in situations in which CSAPTP is  
30 abnormally downregulated and/or in which increased CSAPTP activity is likely to have a beneficial effect. For example, stimulation of CSAPTP activity is desirable in

situations in which a CSAPTP is downregulated and/or in which increased CSAPTP activity is likely to have a beneficial effect. Likewise, inhibition of CSAPTP activity is desirable in situations in which CSAPTP is abnormally upregulated and/or in which decreased CSAPTP activity is likely to have a beneficial effect.

5

### 3. Pharmacogenomics

The CSAPTP molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on CSAPTP activity (e.g., CSAPTP gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders associated with aberrant CSAPTP activity (e.g., proliferative and/or differentiative disorder). In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a CSAPTP molecule or CSAPTP modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a CSAPTP molecule or CSAPTP modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See e.g., Eichelbaum, M., *Clin Exp Pharmacol Physiol*, 1996, 23(10-11):983-985 and Linder, M.W., *Clin Chem*, 1997, 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the



main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drugs target is known (e.g., a CSAPTP protein or CSAPTP receptor of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated

drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., a CSAPTP molecule or CSAPTP modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a CSAPTP molecule or CSAPTP modulator, such as a modulator identified by one of the exemplary screening assays described herein.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of Sequence Listing, figures and all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

## EXAMPLES

**EXAMPLE 1: IDENTIFICATION AND CHARACTERIZATION OF  
HUMAN CSAPTP-1, CSAPTP-2, and CSAPTP-3 cDNAs**

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In this example, the identification and characterization of the genes encoding human CSAPTP-1, CSAPTP-2, CSAPTP-3 and CSAPTP-4 (also referred to as b003g03, b037d02, b067c02 and fchrb018a01, respectively) is described.

10 Isolation of the human CSAPTP cDNA

The invention is based, at least in part, on the discovery of four human genes encoding members of the CSAPTP family. The human CSAPTP family members were isolated from cDNA libraries which were prepared from tissue obtained from subjects suffering from congestive heart failure of ischemic and idiopathic origin. Briefly, a  
15 cardiac tissue sample was obtained from a biopsy of a patient suffering from congestive heart failure. mRNA was isolated from the cardiac tissue and a cDNA library was prepared therefrom using art known methods (described in, for example, *Molecular Cloning: A Laboratory Manual*, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989). Positive clones were isolated either by  
20 examining the top protein blast hit for each sequence, by blasting the libraries against known phosphatases, or by using a computer program that recognizes protein motifs of phosphatases.

The sequences of the positive clones were determined and found to contain open reading frames. The nucleotide sequences encoding the human CSAPTP-1, CSAPTP-2,  
25 CSAPTP-3 and CSAPTP-4 proteins, respectively, are shown in Figures 1, 2, 3 and 15, and are set forth as SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, and SEQ ID NO:10. The full length proteins encoded by these nucleic acids comprise about 173, 263, 209 and 221 amino acids respectively, and have the amino acid sequences shown in Figures 1, 2, 3 and 15, respectively, and set forth as SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8  
30 and SEQ ID NO:11, respectively. The coding region (open reading frame) of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:10 is set forth as SEQ ID NO:3,

SEQ ID NO:6, SEQ ID NO:9 and SEQ ID NO:12, respectively. The clones comprising the entire coding region of human CSAPTP-1, CSAPTP-2, CSAPTP-3 and CSAPTP-4 were deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and  
5 \_\_\_\_\_ 1998, and assigned Accession Nos. \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_ respectively.

#### Analysis of human CSAPTP

A BLAST search (Altschul et al. (1990) *J. Mol. Biol.* 215:403) of the nucleotide  
10 and protein sequences of human CSAPTP-1 revealed that CSAPTP-1 is similar to the following protein: human protein tyrosine phosphatase (Accession No. AAB40597). The CSAPTP-1 nucleic acid sequence is approximately 38.7% identical to that of human protein tyrosine phosphatase, as is shown in Figure 4. The CSAPTP-1 protein sequence is approximately 78.6 % identical (over amino acids 1-173) to that of human protein  
15 tyrosine phosphatase, as is shown in Figure 5.

CSAPTP-2 is similar to the following protein: dual specificity protein phosphatase VHR (Accession No. P51452). The CSAPTP-2 protein is approximately 22.5% identical (over amino acids 1-263) to dual specificity protein phosphatase VHR, as is shown in Figure 6.

20 CSAPTP-3 is similar to the following proteins: dual specificity phosphatase 2 (Accession No. B57126, Q05922), *C. Elegans* protein F28C6.8 (Accession No. Z68315) and *Ovis aries* protein BIIIA3 (Accession No. U60024). The CSAPTP-3 protein is approximately 50 % identical (over amino acids 118-155) and 29% identical (over amino acids 54-97) to mouse dual specificity phosphatase 2. The CSAPTP-3 protein is  
25 approximately 46 % identical (over amino acids 6-20 and 50-64) and 41% identical (over amino acids 20-36) to BIIIA3. The CSAPTP-3 protein is approximately 50 % identical (over amino acids 183-194), 42% identical (over amino acids 92-155) and 39% identical (over amino acids 60-92) to F28C6.8, as is shown in Figure 7.

CSAPTP-4 is similar to the following protein: dual specificity protein phosphatase 3 (Accession No. P51452). The CSAPTP-4 protein is approximately 42% identical (over amino acids 1-221 of SEQ ID NO: 11) to dual specificity protein phosphatase VHR, as shown in Figure 10.

5

#### Tissue Distribution of CSAPTP mRNA

This example describes the tissue distribution of CSAPTP mRNA, as determined by Northern blot hybridizations.

Northern blot hybridizations with the various RNA samples were performed under standard conditions and washed under stringent conditions, i.e., 0.2 X SSC at 65° C. DNA probes corresponding to CSAPTP-1 (Achr003g03), CSAPTP-2 (fchr037d02), CSAPTP-3 (fchr067c02) and CSAPTP-4 (fchr018a01) were used. The DNA was radioactively labeled with <sup>32</sup>P-dCTP using the Prime-It kit (Stratagene, La Jolla, CA) according to the instructions of the supplier. Filters containing human mRNA (MultiTissue Northern I and MultiTissue Northern II from Clontech, Palo Alto, CA) were probed in ExpressHyb hybridization solution (Clontech) and washed at high stringency according to manufacturer's recommendations.

The expression of CSAPTP-1 in various human and rat tissues was assessed; CSAPTP-1 message was detected in human heart and skeletal muscle, where it was highly expressed. CSAPTP-1 mRNA was also found in all rat tissues tested, but was predominantly expressed in rat skeletal muscle, heart, placenta, lung and brain.

CSAPTP-2 message was detected in several human tissues, with highest expression in skeletal muscle and moderate expression in heart and brain. In rat tissues, expression of CSAPTP-2 mRNA was mainly found in skeletal muscle.

CSAPTP-3 message was detected in several human tissues, with highest expression in heart and skeletal muscle, and moderate expression in brain, placenta, kidney and pancreas.

CSAPTP-4 message was detected in human heart and skeletal muscle. CSAPTP-4 mRNA was also expressed in several rat tissues, although most predominantly in rat brain.

## EXAMPLE 2: EXPRESSION OF RECOMBINANT CSAPTP-1 PROTEIN IN C2C12 CELLS

### Preparation of Transgenic C2C12 Cells

5 To express the CSAPTP-1 gene in C2C12 (mouse myoblast) cells, the CSAPTP-1 cDNA was amplified by PCR and cloned into the pcDNA/Amp vector (Invitrogen Corporation (San Diego, CA)). This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. C2C12 cells were  
10 subsequently transfected with the CSAPTP-1-pcDNA/Amp plasmid DNA. Suitable methods for transfecting host cells can be found in Sambrook, J., Fritsch, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

### 15 Expression of Recombinant CSAPTP-1 Protein in Transgenic C2C12 Cells

The expression of the CSAPTP-1 polypeptide in transfected cells is detected by radiolabeling ( $^{35}\text{S}$ -methionine or  $^{35}\text{S}$ -cysteine, available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988).

20 Briefly, the cells are labeled for 8 hours with  $^{35}\text{S}$ -methionine (or  $^{35}\text{S}$ -cysteine). The culture medium is then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). The CSAPTP-1 polypeptide is immunoprecipitated from the cell lysate and the culture medium and analyzed by SDS-PAGE.

25

### Phenotype of C2C12 Cells Expressing CSAPTP-1

C2C12 cells are myoblasts, and, upon confluency, are able to rapidly differentiate to form contractile myotubes. This myotube formation is known to be stimulated by the utilization of horse serum (as opposed to fetal bovine serum) in the  
30 medium. To determine whether the expression of the CSAPTP-1 gene in C2C12 cells impacts the ability of the cells to differentiate into myotubes, both untransfected control

C2C12 cells and a stably transfected CSAPTP-1-containing C2C12 cell line were grown for 5 days in standard growth medium containing 20% fetal bovine serum. After 5 days the medium was replaced with medium containing 2% horse serum, and the ability of the cells to fuse into myotubes was assessed. The untransfected control cells elongated and fused, forming myotubes. In contrast, the CSAPTP-1 containing cells displayed a reduced ability to fuse and formed many fewer higher-order myotube structures.

### EXAMPLE 3: GENERATION OF CSAPTP-1 TRANSGENIC ANIMALS

10 An N-terminal His-tagged CSAPTP-1 cDNA was ligated to the cardiac-specific alpha-myosin heavy chain promoter and the human growth hormone polyadenylation signal sequence using art known techniques. This construct was microinjected into the male pronucleus of fertilized mouse eggs, which were subsequently implanted into a pseudopregnant foster mother. Founder mice were bred with non-transgenic cohorts for the propagation of individual lines.

The presence of the CSAPTP-1 transgene in transgenic mice was confirmed by Southern analysis of genomic DNA. Briefly, 10 micrograms of mouse genomic DNA was digested with EcoRI or BamHI or XbaI (New England Biolabs), electrophoresed in a 0.8% agarose gel and transferred to a nylon membrane (Genescreen, DuPont).  
20 Membranes were hybridized with a <sup>32</sup>P-labeled cDNA probe specific for the CSAPTP-1 sequence. Results confirmed that both of the founder mice 03645-4.7F and 03650-3.8F were found to have significant levels of the CSAPTP-1 transgene, whereas transgene expression was not visible in the wild-type mouse genomic DNA.

The expression of the CSAPTP-1 gene in the transgenic mice was also assessed by Northern analysis. Total RNA from both wild-type and transgenic mice was isolated using the guanidinium isothiocyanate/acid phenol procedure (Chomczynski, (1987) Anal. Biochem. 162: 156-159). Northern blots (Sambrook (1989) *ibid*) were performed with 20 micrograms of total RNA from the heart tissue fractionated in a 1.5% agarose/formaldehyde gel and blotted onto a nylon membrane (Genescreen, DuPont).  
30 Hybridization and washing were performed as recommended by the supplier. Briefly, membranes were probed using a <sup>32</sup>P-labeled probe for CSAPTP-1, applied in 50%

formamide hybridization solution at 42°C for 18h. The results demonstrated that there is a significant amount of mRNA for the transgene in both the 03645-4.7F and 03650-3.8F founder mice, whereas message was undetectable in the wild type mouse.

The phenotypes of the transgenic mice were also assessed. The transgenic animals showed a 37% increase in heart/body ratio as compared to wild type mice, indicating that the heart was enlarged. Similarly, a significant number of the cardiomyocytes in transgenic mouse hearts were hypertrophied. An examination of the heart tissue from transgenic mice demonstrated the presence of calcification, similar to that seen in advanced stages of cardiomyopathy.

10

**EXAMPLE 4:        ASSESSMENT OF THE EFFECT OF CSAPTP-1  
                         EXPRESSION ON GENE EXPRESSION**

To determine whether the expression of CSAPTP-1 had an effect on the expression of certain other genes of interest, (e.g., oncogenes, tumor suppressors, cell cycle regulators, transcription factors, cell surface antigens, or intracellular signal transduction modulators and effectors, or those genes involved in stress response, ion channels and transport, apoptosis, DNA repair, receptors, or cell-cell communications), a cDNA expression array was performed using an cDNA expression array kit (Clontech) according to the manufacturer instructions. In this procedure, labeled cDNA probe mixtures were synthesized by reverse transcription from the RNA population of whole heart tissue from both a wild type mouse and from mice containing the CSAPTP-1 transgene. Each labeled probe mixture was hybridized to an array of cDNAs for the gene of interest, the arrays were subjected to a high-stringency wash, and the hybridized labeled probes were detected and quantified by phosphorimaging. The gene levels were normalized to GADPH expression.

The expression of a number of genes was different between the wild-type and the CSAPTP-1 transgenic mice. The results of an additional cDNA expression array experiment utilizing probes synthesized from control C2C12 cells and C2C12 cells containing the CSAPTP-1 transgene also indicated that a number of genes were affected by the expression of CSAPTP-1 (see Figure 8). A 7.5-fold decrease in the presence of

30



the transcript for the DB-1 gene was observed in C2C12 cells expressing the CSAPTP-1 gene as compared to control cells. DB-1 is a zinc finger transcription factor which was first cloned by its affinity to an element *cis* to the IL-3 promoter (nucleotides -76 to -47), a region which is rich in CT/GC. This region is known to bind Egr1 and Egr2, which, in the presence of TPA, transactivates the IL-3 promoter. The association of DB-1 and Egr1 or Egr2 has not been demonstrated to transactivate the IL-3 promoter. DB-1 is ubiquitously expressed, and has been shown to interact with both Tax and with prenylated RhoB. Upon binding to Tax, DB-1 is able to synergistically activate the IL-3 promoter. The interaction of DB-1 with prenylated RhoB has been implicated in actin regulation, cell growth (e.g., transformation), gene activation (e.g., SREs), and cell adhesion and motility (e.g., integrins).

In addition, a decrease in transcription of the L1CAM and CD27 genes was detected in the CSAPTP-1- transgenic C2C12 cells in comparison with wild-type cells. In contrast, the transcription of the PLA2, caspase, thymosin, and Hox7 genes was increased in the CSAPTP-1 transgenic C2C12 cells as compared to control C2C12 cells.

#### **EXAMPLE 5:           EXPRESSION OF RECOMBINANT CSAPTP PROTEIN IN BACTERIAL CELLS**

In this example, CSAPTP is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, CSAPTP is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-CSAPTP fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

### Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following

5 claims.

What is claimed:

1. An isolated nucleic acid molecule selected from the group consisting of:
  - a) a nucleic acid molecule comprising a nucleotide sequence which  
5 is at least 38.7%, 50%, 50% or 50% homologous to a nucleotide sequence of  
SEQ ID NO:1 or 3, SEQ ID NO:4 or 6, SEQ ID NO:7 or 9, or SEQ ID NO:10 or  
12, respectively, the DNA insert of the plasmid deposited with ATCC as  
Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, or a complement thereof;
  - b) a nucleic acid molecule comprising a fragment of at least 994,  
10 626, 531 or 241 nucleotides of a nucleic acid comprising the nucleotide sequence  
of SEQ ID NO:1 or 3, SEQ ID NO:4 or 6, SEQ ID NO:7 or 9, or SEQ ID NO:10  
or 12, respectively, the DNA insert of the plasmid deposited with ATCC as  
Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, or a complement thereof;
  - c) a nucleic acid molecule which encodes a polypeptide comprising  
15 an amino acid sequence at least 79%, 23%, 24% or 42% homologous to the  
amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID  
NO:11, respectively, or an amino acid sequence encoded by the DNA insert of  
the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_,  
or \_\_\_\_\_;
  - d) a nucleic acid molecule which encodes a fragment of a  
20 polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID  
NO:5, SEQ ID NO:8 or SEQ ID NO:11, or the polypeptide encoded by the DNA  
insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_,  
\_\_\_\_\_, or \_\_\_\_\_, wherein the fragment comprises at least 15 contiguous amino  
25 acid residues of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ  
ID NO:8 or SEQ ID NO:11, or the polypeptide encoded by the DNA insert of the  
plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or  
\_\_\_\_\_; and
  - e) a nucleic acid molecule which encodes a naturally occurring  
30 allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID  
NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or an amino acid

sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:12 under stringent conditions.

2. The isolated nucleic acid molecule of claim 1 which is selected from the group consisting of:

a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12 or the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, or a complement thereof; and

b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_.

3. The nucleic acid molecule of claim 1 further comprising vector nucleic acid sequences.

4. The nucleic acid molecule of claim 1 further comprising nucleic acid sequences encoding a heterologous polypeptide.

5. A host cell which contains the nucleic acid molecule of claim 1.

6. The host cell of claim 5 which is a mammalian host cell.

7. A non-human mammalian host cell containing the nucleic acid molecule of claim 1.

8. An isolated polypeptide selected from the group consisting of:

5 a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or the polypeptide encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_;

10 b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:12 under stringent conditions; and

15 c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 38.7%, 50%, 50% or 50% homologous to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1 or 3, SEQ ID NO:4 or 6, SEQ ID NO:7 or 9, or SEQ ID NO:10 or 12, respectively, the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_.

20 d) a polypeptide comprising an amino acid sequence which is at least 79%, 23%, 24% or 42% homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, respectively, or the polypeptide encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_.

30

9. The isolated polypeptide of claim 8 comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_.

5

10. The polypeptide of claim 8 further comprising heterologous amino acid sequences.

11. An antibody which selectively binds to a polypeptide of claim 8.

10

12. A method for producing a polypeptide selected from the group consisting of:

a) a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_;

15

b) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_ wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_; and

20

c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 or SEQ ID NO:11, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID

25

30

NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:12 under stringent conditions;

comprising culturing the host cell of claim 5 under conditions in which the nucleic acid molecule is expressed.

5

13. A method for detecting the presence of a polypeptide of claim 8 in a sample comprising:

- a) contacting the sample with a compound which selectively binds to the polypeptide; and
- 10 b) determining whether the compound binds to the polypeptide in the sample to thereby detect the presence of a polypeptide of claim 8 in the sample.

14. The method of claim 13, wherein the compound which binds to the polypeptide is an antibody.

15

15. A kit comprising a compound which selectively binds to a polypeptide of claim 8 and instructions for use.

20 16. A method for detecting the presence of a nucleic acid molecule in claim 1 in a sample comprising:

- a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and
- 25 b) determining whether the nucleic acid probe or primer binds to a nucleic acid molecule in the sample to thereby detect the presence of a nucleic acid molecule of claim 1 in the sample.

25

17. The method of claim 16, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

30

18. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of claim 1 and instructions for use.

19. A method for identifying a compound which binds to a polypeptide of claim 8 comprising:

- a) contacting the polypeptide, or a cell expressing the polypeptide with a test compound; and
- b) determining whether the polypeptide binds to the test compound.

20. The method of claim 19, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

- a) detection of binding by direct detection of test compound/polypeptide binding;
- b) detection of binding using a competition binding assay; and
- c) detection of binding using an assay for CSAPTP activity.

21. A method of modulating the activity of a polypeptide of claim 8 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

22. A method for identifying a compound which modulates the activity of a polypeptide of claim 8 comprising:

- a) contacting a polypeptide of claim 8 with a test compound; and
- b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.



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## FIG. 1

## DNA SEQUENCE:

GTCGACCCACGCGTCCGTGGGTTTTCTTTTTTAATTATCCAAACAGTGG  
GCAGCTTCCTCCCCCACACCCAAGTATTTGCACAATATTTGTGCGGGGT  
ATGGGGGTGGGTTTTTAAATCTCGTTTCTCTTGGACAAGCACAGGGATC  
TCGTTCTCCTCATTTTTTTGGGGGTGTGTGGGGACTTCTCAGGTCGTGTC  
CCCAGCCTTCTCTGCAGTCCCTTCTGCCCTGCCGGGCCCCGTCGGGAGGC  
GCCATGGCTCGGATGAACCGCCCCGGCCCCGGTGGAGGTGAGCTACAAAC  
ACATGCGCTTCCTCATCACCCACAACCCCAACGCCACGCTCAGCAC  
CTTCATTGAGGACCTGAAGAAGTACGGGGCTACCACTGTGGTGCGTGTG  
TGTGAAGTGACCTATGACAAAACGCCGCTGGAGAAGGATGGCATCACCG  
TTGTGGACTGGCCGTTTGACGATGGGGCGCCCCCGCCCGGCAAGGTAGT  
GGAAGACTGGCTGAGCCTGGTGAAGGCCAAGTTCTGTGAGGCCCCCGGC  
AGCTGCGTGGCTGTGCACTGCGTGGCGGGCCTGGGCCGGGCTCCAGTCC  
TTGTGGCGCTGGCCCTTATTGAGAGCGGGATGAAGTACGAGGACGCCAT  
CCAGTTCATCCGCCAGAAGCGCCGCGGAGCCATCAACAGCAAGCAGCTC  
ACCTACCTGGAGAAATACCGGCCCAAACAGAGGCTGCGGTTCAAAGACC  
CACACACGCACAAGACCCGGTGTGCGTTATGTAGCTCAGGACCTTGGC  
TGGGCCTGGTCGTATGTAGGTCAGGACCTTGGCTGGACCTGGAGGCCC  
TGCCCAGCCCTGCTCTGCCCAGCCCAGCAGGGGCTCCAGGCCTTGGCTG  
GCCCCACATCGCCTTTTCTCCTCCCCGACACCTCCGTGCACTTGTGTCCGA  
GGAGCGAGGAGCCCCCTCGGGCCCTGGGTGGCCTCTGGGCCCTTTCTCCT  
GTCTCCGCCACTCCCTCTGGCGGGCGCTGGCCGTGGCTCTGTCTCTCTGA  
GGTGGGTGCGGGCGCCCTCTGCCCGCCCCCTCCACACCAGCCAGGCTGG  
TCTCCTCTAGCCTGTTTGTGTGGGGTGGGGGTATATTTTGTAACCACT  
GGGCCCCCAGCCCCCTCTTTTGCGACCCCTTGTCCTGACCTGTTCTCGGC  
ACCTTAAATTATTAGACCCCGGGGCAGTCAGGTGCTCCGGACACCCGAA  
GGCAATAAAAACAGGAGCCGTGAAAAAAAAAAAAAAAAAAAAAAAAAAAA  
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGGCGGCCGN

## AMINO ACID SEQUENCE:

MARMNRPAPEVVS YKHMRF LITHNPT NATLST FIEDLK KYGATT VVRVCE VTYDKT  
PLEKDGITVVDWPFDDGAPPPGKVVEDWLSLVKAKFCEAPGSCVAVHCVAGLGRAP  
VLVALALIESGMKYEDIAIQFIRQKRRGAINSKQLTYLEKYRPKQRLRFKDPHTHKT  
RCCVM

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## FIG. 2

## DNA SEQUENCE:

GTGACCCACGCGTCCGGCGGCTCCTCTACACAGGCAAGACAGCCTGTAACCATGC  
CGACGAGGTCTGGCCAGGCCTCTATCTCGGAGACCAGGACATGGCTAACAACCGCC  
GGGAGCTTCGCCGCCTGGGCATCACGCACGTCCTCAATGCCTCACACAGCCGGTGG  
CGAGGCACGCCCAGGCCTATGAGGGGCTGGGCATCCGCTACCTGGGTGTTGAGGC  
CCACGACTCGCCAGCCTTTGACATGAGCATCCACTTCCAGACGGCTGCCGACTTCA  
TCCACCGGGCGCTGAGCCAGCCAGGAGGGAAGATCCTGGTGCATTGTGCTGTGGGC  
GTGAGCCGATCCGCCACCCTGGTACTGGCCTACCTCATGCTGTACCACCACCTTAC  
CCTCGTGGAGGCCATCAAGAAAGTCAAAGACCACCGAGGAGAGGCCGAGCCCCAGG  
CCACTGTCACTCTTTGTGGGAGGGGACGGGGAGTGAGGTTGGGCAGTGTGGTGGAT  
GGGCACCCAGGAAGGGTTGACCAGGGAAGGAGGCAGCTAGGCTGTAGATGGAAGAT  
GGTCTTGGGATTCGAACACCGCTGGGATCTGGCCAGGGTGCTCCCTGGGATTCA  
GTCCCTTCCCCTCTTTGTGCCCAAGTGTTCCTCTCTCCCTCACCAAAAACAAA  
AGGGCCATCTCTGCCCCTGCACTTTGTGCAGAAAGTCAGGGATACGGCAAGCATGA  
ATGCAATGGTGTAGAGTTGTGTGAAACCCCTAGCATAGAGACAGACAGCGAAGAGA  
TGGTGTGAAAAGCTTGCAGAACCAGACAGAGAACCCACAGACTTTCCACTCCAAG  
CACAGGAGGAGGTAGCTAGCGTGTGAGGGTTGGCACTAGGCCCACGGCTGCTGCTT  
GGGCCAAAACATACAGAGGTGCATGGCTGGCAGTCTTGAAATTGTCACTCGCTTA  
CTGGATCCAAGCGTCTCGAGGATAAATAAAGATCATGAAAAAAAAAAAAAAAAAAGG  
GCGGCCGC

## AMINO ACID SEQUENCE:

VDPRVRRLLYTGKTACNHADEVWPGLYLGDQDMANNRRELRLGLITHVLNASHSRW  
RGTPEAYEGLGIRYLGVEAHDSPAFDMSIHFTAADFIHRALSQPGGKILVHCAVG  
VSRSATLVLAYIMLYHHLTLVEAIKKVKDHRGEAEPQATVTLCGRGRGVRLGSVVD  
GHPGRVDQGRRQLGCRWKMLVGFHRWDLARVLPGLHSPFPSLCPSVSLSPSPKTK  
RAISAPALCAESQYGKHECNGVELCETPSIETDSEEMV

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## FIG. 3

## DNA SEQUENCE:

TNGGATCGAMCNSGCGTCCGGGCGGCCCCGCGCTGCTGGAGGCCGGCCTGGCGCG  
GGTGCTCTTCTACCCGACGCTGCTCTACACCCTGTTCCGCGGGAAGGTGCCGGGT  
CGGGCGCACCGGGACTGGTACCACCGCATCGACCCACCGTGCTGCTGGGCGCGC  
TGCCGTTGCGGAAGCTTGACGCGCCAGCTGGTACAGGACGAGAACGTGCGCGGGG  
TGATCACCATGAACGAGGAGTACGAGACGAGGTTCTGTGCAACTCTTCACAGGA  
GTGGAAGAGACTAGGAGTCGAGCAGCTGCGGCTCAGCACAGTAGACATGACTGGG  
ATCCCCACCTTGGAACAACCTCCAGAAGGGAGTCCAATTTGCTCTCAAGTACCAGT  
CGCTGGGCCAGTGTTGTTTACGTGCATTGTAAGGCTGGGCGCTCCAGGAGTGCCAC  
TATGGTGGCAGCATACTGATTCAGGTTGCACAAATGGGAGTCCAGAGGAGGCTG  
NNNGAGCCATCGCCCAAGATCCGGGTCATACATCCACATCAGGCCTGGCCAGCTT  
GGATGTTCTTAAAGAGTTCACAAAGCAGATTACTGCACSGGCMACAAAGGATGG  
GACTTTTGKCATTTCAAAGACATGATGTATGGGGATTAGAAAGAACTCAAGACAC  
TCCTGCTTGATACAGAACAAAAAGAGCTTAACAGGACCAACAGGGCTTAAGCCCA  
GACTTGACGTAACAGAAATGTGCCAATAGGTAATAGGTAATTTTTCTTTCTCTGA  
CTTGTTTTGTTTTCTNAAATGGCACTGTTGAANNANTNTNNCTC

## AMINO ACID SEQUENCE:

XDRXXVRAAPRCWRPAWRGCSSTRRCSTPCSAGRCRVGRTGTGTTASTPPCCWAR  
CRGSLTRQLVQDENVRGVITMNEEYETRFLCNSSQEWKRLGVEQLRLSTVDMTG  
IPTLDNLQKGVQFALKYQSLGQCVYVHCKAGRSRSATMVAAYLIQVAQMGVQRRL  
XEPSPKIRVIHPHQAWPAWMFLKSSTKQITAXXTKDGTFXISK

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## FIG. 4-1

ALIGN calculates a global alignment of two sequences

version 2.0 Please cite: Myers and Miller, CABIOS (1989)  
 > Achrb003g03cons - Vector Check 1315 aa vs.  
 > Genbank U48296 - Human protein tyrosine phosphatase 2200 aa  
 scoring matrix: paml20.mat, gap penalties: -12/-4  
 38.7% identity; Global alignment score: 49

```

              10          20          30          40
inputs -----GTCGACC---CACG---CGTCCG-TGGGTTTTCTTTTAAATTATCCAAACAGT
              10          20          30          40          50
              CGGGATTACTGCCAGGCACAGCACGACCTCTATGCAGACAAGTG-AACTGTAGAACTGA

              50          60          70
inputs GGGCAGCT--TCCT-----CCCCCA-----CACCCAAGTATT-----
              60          70          80          90          100          110
              TTACTGCTCCACCAAGAAGCCCCCATAAGAGTGGTTATCCTGGACACAGAAGTGTGAAT

              80          90          100          110
inputs TG-----CACAATA-TTTGTGC--GGGGTATGG---GGGTGGGTTTTT---AAATC---
              120          130          140          150          160          170
              TGAAATCCACAGAGCATTTTACAAGAGTTCTGACCTGGATGGGGTAAACCTCAGTGCCT

              120          130          140          150          160          170
inputs TCGTTTCTCTTGGACA-----AGCACAGGGATCTCGTTCTCCT-CAT-TTTTTGGGGGTGT
              180          190          200          210          220          230
              TCTTTTCTGTGGCCTCAGTATTACTGGATTGAAGAATTGCTGCTTCTTGTAGGAGGTT

              180          190          200
inputs GTGGGGACTTCTCAGGTCGTG-----TC---CCCA--GC-CT-----TCT-----
              240          250          260          270          280          290
              CATTTCACTTATCATTACTTACAACCTCATACTCAAAGCACTGAGAATTTCAAGTGGAGT

              210          220
inputs -----CTGCAGTCCCTT--CTGCC--CTG-----
              300          310          320          330          340          350
              ATATTGAAGTAGACTTCAGTTTCTTTGCATCATTCTGTATTCAATTTTTTTAATTATTT

              230          240          250          260          270
inputs CCGGGCCC-GTCGGGAG-----GC-----GCCATGGCTCGGATGAACCGCCCGCCCCG
              360          370          380          390          400          410
              CATAACCCTATTGAGTGTTTTTAATAAATGCTCGAATGAACCGCCAGCTCCT

              280          290          300          310          320          330
inputs GTGGAGGTGAGCTACAAACACATGCGCTTCTCATCACCCACAACCCACCAACGCCACG
              420          430          440          450          460          470
              GTGGAAGTCACATACAAGAACATGAGATTCTTATTACACACAATCCAACCAATGCGACC

              340          350          360          370          380          390
inputs CTCAGCACCTTCATTGAGGACCTGAAGAAGTACGGGGCTACCACTGTGGTGCGTGTGTG

```

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**FIG. 4-2**

[illegible]

**FIG. 4-3**

```

      850      860      870
inputs -----C-TCTGCCCA-----GCCCAGCAGGGGCTCCAGGC-----
      :   : : : : :   :   : : : : : : : :
      TTGGGTTACAGTCAACCTATTTGGATACTTGGCAAAGATTCTTGCTGTCAGCATATAAA
1080      1090      1100      1110      1120      1130

      880      890
inputs -----CTTGGC-----TGGCC-----CCACATC--GC-----
      : : : :   : : : :   : : : :   : :
      ATGTGCTTGTCAATTGTATCAATTGACCTTTCCCAAATCATGCAGTATTGAGTTATGAC
1140      1150      1160      1170      1180      1190

      900
inputs -----CTTTTCC-----TC-----
      : : : : :   : :
      TTGTTAAATCTATTTCCCATGCCAGAATCTTATCAATACATAAGAAATTTAGGAAGATTAG
1200      1210      1220      1230      1240      1250

      910      920      930
inputs -----CCCACAC-----CT-----CCGTGCACTTGTG---TCCG
      : : : : :   : :   : : : : :   : :
      GTGCCAAAATACCCAGCACAACTTGTATATTTTTAGTACCATACAGAAGTAAATCCC
1260      1270      1280      1290      1300      1310

      940      950
inputs AGGAGCGAGGAGC-----CCCTCGGGC-----CCTGGGT-
      : : : : :   : : : : :   : : : :
      AGGAACATGAACACTAGACCTTATGTGGTTTATTCCTTCAATCATTTCAAACATTGAAA
1320      1330      1340      1350      1360      1370

      960      970      980      990
inputs ---GGCCT-C-TGG-----GCCCTTTCTCCT-----GTCTCCGCCACTC---CC
      : : : : :   : :   : : : :   : : : :   : : : :
      GTAGGGCCTACATGGTTATTTGCCTGCTCACTTTATGTTTACATCTCCACATTATAC
1380      1390      1400      1410      1420      1430

      1000      1010      1020      1030
inputs TCTG-GCGGC-----GCT-GGCCGTGG-----CTCTGTCTCTCTGAGG
      : . : : :   : : : : :   : : : : :   : : : :
      AATATACGTCAGGTTTGCTTAACCATTGATTTTTTTTTTTTTTACCAAGTCTTACAGTGA
1440      1450      1460      1470      1480      1490

      1040      1050
inputs TGGGT---CGGGCG-CC-----CTC-----TGC-----CCGCCC-----
      : . :   : : :   : :   : :   : :   : :
      TTATTTTACGTGTTTCCATGTATCTCACTTTGTGCTGTATTAAAAAACCTCCATTTTGA
1500      1510      1520      1530      1540      1550

      1060      1070
inputs ---CCTC-----C-----CACA---C-----CAG-----CCAGGC---
      : . :   : : : :   : :   : :   : :   : :
      AAATCTACGTTGTACAGAAGCACATGTCTTTAATGTCTTCAGACAAAAAGCCTTACATT
1560      1570      1580      1590      1600      1610

      1080      1090      1100
inputs -----TGGTCTC-CTCTA-----GCCTGTTTGTGTG-GGGT

```

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## FIG. 4-4

```
      :: : : ::::.      ::::. .... ::::
AATTTAATGTTTGCACCTCTGAGGTGCAACTTAACAGGGAGGGCCTGAGAAAAGAATGGGA
1620      1630      1640      1650      1660      1670

      1110      1120      1130
inputs GGGGG-----TATATTTT-----GTAACCA-----CTGG-GCCCCCA-----
      ::::.      ::::.      ::::.      :: : : :
GGGGGCTATTAATTATTTTGTAGCAAATGTTGCCCTTGTCTTGTGCAAACATGTAGAAT
1680      1690      1700      1710      1720      1730

      1140
inputs --GCCC-----CT-----C
      :: : : :
ATGCTCTTTAATCTAGTAAATATTTTTTTTAAAGGTAGAGATGCTTTGTTATTGTAATC
1740      1750      1760      1770      1780      1790

      1150      1160      1170
inputs TTTTGCAGCCC-----CTTGTCTGA-----CCTGTTCT-----CGG-----CACC
      ::::. : : : : : : : : : : : : : : :
ATAAACTTCTGAAATTCTTGTAAATTTTCCCATACTTATCAGAAGTGTGTTTACCAAC
1800      1810      1820      1830      1840      1850

      1180      1190
inputs TTAAATTA-TTAGA-----CC-CC-----
      ::::. : : : : : : : : : : :
TTATTTTGTGTTGAAAGTGTGATTTTTTTTTTCTTCCCAACCTCTCTTGCAAAAAAAGA
1860      1870      1880      1890      1900      1910

      1200      1210
inputs ---GGG-----GCAG---TCAG-----GTGCT---
      : : : : : : : : : : :
AATGGGTTTCTGCTAATGAATTGAGCAGAGATCTAATATTTTATATGCCTTTTGAGCTGT
1920      1930      1940      1950      1960      1970

      1220      1230      1240
inputs -----C-CCGAAGGCAATAA--AACAGGAG-----CC
      : : : : : : : : : : :
TGTATTAATGTTAGTTCAACCATATATTTATACTGTCTGGGGATGTGTGTTATAGTTCT
2040      2050      2060      2070      2080      2090

      1250      1260      1270      1280
inputs GTGAAAAAAAAAAAAAAAAA-----AAAAAAAAAAAAAAAAAAAAAAAAA
      ::::. : : : : : : : : : : :
GTGGGAGAAATAATTTGTTCAGTGTTCACCAGCTTGTAAAACTTAGTGCAGAGAGCTGAA
2100      2110      2120      2130      2140      2150

      1290      1300      1310
inputs AAAAAAAAAAAAAAAAAAGGGCG-GC-----CGN-----
      : : : : : : : : : : :
ACATCTAAATAAATAATGACATGCATTTATCATCATTGAAA
2160      2170      2180      2190      2200
```

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## FIG. 5

ALIGN calculates a global alignment of two sequences  
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989)  
 >PTPCAAXI 173 aa  
 > fchr003g03  
 scoring matrix: pam120.mat, gap penalties: -12/-4  
 78.6% identity; Global alignment score: 776

inputs	MARMNRPA	PAPVEV	TYKNNR	FLITHN	PTNATLN	KFIEEL	KKYGV	TTIVR	VCEATY	DTTL	VEK	60
	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
	MARMNRPA	PAPVEV	SYKNNR	FLITHN	PTNATLN	STFIED	LKKYG	ATTVVR	VCEVYD	KTPLEK		60
	10	20	30	40	50	60						
inputs	EGIHVLD	WPFD	DGAPPS	NIQIV	DDWLS	LVKIK	IFREE	PGCCIA	VHCV	AGLGR	PVLVAL	ALI
	...	...	...	...	...	...	...	...	...	...	...	...
	DGITVVD	WPFD	DGAPPS	NIQIV	DDWLS	LVKIK	IFREE	PGCCIA	VHCV	AGLGR	PVLVAL	ALI
	70	80	90	100	110	120						
inputs	EGMKYED	AVQFIR	KRRGAF	NSKQL	LYLEK	YRPM	RLRF	KDSNG	HNNCCI	Q		
	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	ESGMKYED	AVQFIR	KRRGAF	NSKQL	LYLEK	YRPM	RLRF	KDSNG	HNNCCI	Q		
	130	140	150	160	170							



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## FIG. 6

ALIGN calculates a global alignment of two sequences  
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989)  
 > b037d02 prot 263 aa vs.  
 > SwissProt P51452 - DUAL SPECIFICITY PROTEIN PHO 185 aa  
 scoring matrix: pam120.mat, gap penalties: -12/-4  
 22.5% identity; Global alignment score: 205

```

              10          20          30          40
inputs  ----VDPRVRL--LYTGKTAC---NHA-DEVWPGLYLGDQDMANNRRELRRRLGITHVL
          .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .
MSGSFELSVQDLNDLLSDGSGCYSLPSQPCNEVTPRIYVGNASVAQDIPKLQKLGITHVL
              10          20          30          40          50          60

              50          60          70          80          90          100
inputs  NASHSRW----RGTPEAYEGLGIRYLGVEAHDSPAFDMSIHFQTAADFIHRALSQPGGKI
          .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .
NAAEGRSFMHVNTNANFYKDSGITYLGIKANDTQEFNLSAYFERAADFIDQALAQKNGRV
              70          80          90          100          110          120

              110          120          130          140          150          160
inputs  LVHCAVGVSRSATLVLAYLMLYHHLTLVEAIKKVKDHRGEAEPQATVT-LCG-RGRGVRL
          .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .
LVHCREGYRSPTLVIAYLMMRQKMDVKSALSIVRQNREIGPNDGFLAQLCQLNDRLAKE
              130          140          150          160          170          180

              170          180          190          200          210          220
inputs  GSVVDGHPGRVDQRRQLGCRWKMLGFEHRWDLARVLPGIHSPFSLCPSVSLSPSKT
          :  .
GKLKP-----

              230          240          250          260
inputs  KRAISAPALCAESQGYGKHECNGVELCETPSIETDSEEMV
          -----

```

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## FIG. 7

ALIGN calculates a global alignment of two sequences  
 version 2.0u Please cite: Myers and Miller, CABIOS (1989)  
 > Genbank 1657672 | Z68315 Caenorhabditis elegans 150 aa vs.  
 > Achrb067c02.aa 202 aa  
 scoring matrix: paml20.mat, gap penalties: -12/-4  
 24.0% identity; Global alignment score: -63

```

                                10
inputs -----MPFRSMKDEL
                                .....
VRAATALLEAGLARVLFYPTLLYTLFRGKVPGRAHRDWYHRIDPTVLLGALPLRSLTRQL
      10      20      30      40      50      60
      20      30      40      50      60      70
inputs IQENVGGVVCCTEEFELKAAMNAMREVDWKNEGVEFFAVPMKDFTGTAPRAEINEAVEF
      ..:::..:.....:..:..:..:..:..:..:..:..:..:..:..:..:
VQDENVRGVITMNEEYETRFLCNSSQE--WKRLGVEQLRLSTVDMTGIPTLNLOKGVQF
      70      80      90      100     110
      80      90      100     110     120     130
inputs IESVASKGKTVYVHCKAGRTRSATVATCYLMKSRNWMNSVAVWEFLKDKRHQVLLRNAHWR
      :..:.....:.....:.....:.....:.....:.....:.....:
ALKYQSLGQCQVYVHCKAGRSRSATMVAAYLIQVHKWSPEEAVRAIAKIRSYIHIRPGQLD
    120     130     140     150     160     170
      140     150
inputs TVNEYRRFLDSNSSSTG----SSN
      .....:..
      VLKEFHKQITARATKDGTFVISK
    180     190     200

```

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# FIG.8

## Quantitative Results of cDNA Array

C2C12 Control		C2C12 - CSAPTP - 1 Pooled	
<u>Genes</u>	<u>Density</u>	<u>Density</u>	<u>Normalized to GAPDH</u>
GAPDH	654	GAPDH	1.00
PLA2	681	PLA2	1.10
L1CAM	584	L1CAM	0.69
CD27	535	CD27	0.69
Caspase	780	Caspase	1.80
Thymosin	847	Thymosin	1.45
Hox7	494	Hox7	0.85
DB - 1	2255	DB - 1	0.49

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## FIG. 9-1

1	5	10	15	48
tgt tcc gct cag gca gag tcc tgc ccc tgc acc cac tcc ccc att ccc				
Cys Ser Ala Gln Ala Glu Ser Cys Pro Cys Thr His Ser Pro Ile Pro				
2	7	12	17	
ggc ccc agg cca tgc ccc agg atg gac tca ctg cag aag cag gac ctc				96
Gly Pro Arg Pro Cys Pro Arg Met Asp Ser Leu Gln Lys Gln Asp Leu				
3	9	14	19	
cgg agg ccc aag atc cat ggg gca gtc cag gca tct ccc tac cag ccg				144
Arg Arg Pro Lys Ile His Gly Ala Val Gln Ala Ser Pro Tyr Gln Pro				
4	11	16	21	
ccc aca ttg gct tcg ctg cag cgc ttg ctg tgg gtc cgt cag gct gcc				192
Pro Thr Leu Ala Ser Leu Gln Arg Leu Leu Trp Val Arg Gln Ala Ala				
5	13	18	23	
aca ctg aac cat atc gat gag gtc tgg ccc agc ctc ttc ctg gga gat				240
Thr Leu Asn His Ile Asp Glu Val Trp Pro Ser Leu Phe Leu Gly Asp				
6	15	20	25	
gcg tac gca gcc cgg gac aag agc aag ctg atc cag ctg gga atc acc				288
Ala Tyr Ala Ala Arg Asp Lys Ser Lys Leu Ile Gln Leu Gly Ile Thr				
7	17	22	27	
cac gtt gtg aat gcc gct gca ggc aag ttc cag gtg gac aca ggt gcc				336
His Val Val Asn Ala Ala Ala Gly Lys Phe Gln Val Asp Thr Gly Ala				

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## FIG. 9-2

aaa ttc tac cgt gga atg tcc ctg gag tac tat ggc att gag gcg gac lys phe tyr arg gly met ser leu glu tyr tyr gly ile glu ala asp 115 120 125 384
gac aac ccc ttc ttc gac ctc agt gtc tac ttt ctg cct gtt gct cga asp asn pro phe phe asp leu ser val tyr phe leu pro val ala arg 130 135 140 432
tac atc cga gct gcc ctc agt gtt ccc caa ggc cgc gtg ctg gta cac tyr ile arg ala ala leu ser val pro gln gly arg val leu val his 145 150 155 160 480
tgt gcc atg ggg gta agc cgc tct gcc aca ctt gtc ctg gcc ttc ctc cys ala met gly val ser arg ser ala thr leu val leu ala phe leu 165 170 175 528
atg atc tat gag aac atg acg ctg gta gag gcc atc cag acg gtg cag met ile tyr glu asn met thr leu val glu ala ile gln thr val gln 180 185 190 576
gcc cac cgc aat atc tgc cct aac tca ggc ttc ctc cgg cag ctc cag ala his arg asn ile cys pro asn ser gly phe leu arg gln leu gln 195 200 205 624
gtt ctg gac aac cga ctg ggg cgg gac acg ggg cgg ttc tgatctggca val leu asp asn arg leu gly arg asp thr gly arg phe 210 215 220 673

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## FIG. 9-3

ggcagccagg atccctgacc cttggcccaa cccaccagc ctggccctgg gaacagcagg 733  
ctctgctgtt tctagtgacc ctcagatgta aacagcaagt gggggctgag gcagaggcag 793  
ggatagctgg gtggtgacct cttagcgggt ggatttcctt gacccaattc agagatttctt 853  
tatgcaaaaag tgagttcagt ccatctcata taataaataa ttcantngtc ataaaaaaaa 913  
aaaaaaaaag gcgcc 928

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## FIG. 10

GAP of: FrGcgManager\_77\_MOASSfTL\_ check: 6769 from: 1 to: 221

csaptp4pro (analysis only) - Import - complete

to: FrGcgManager\_77\_NOA0mprsU check: 3219 from: 1 to: 185

P51452 in SwissProt

Symbol comparison table: /prod/ddm/sequal/BLAST/matrix/aa/BLOSUM62  
CompCheck: 1102

Matrix made by matblas from blosum62.iiij

Gap Weight:	12	Average Match:	2.778
Length Weight:	4	Average Mismatch:	-2.248

Quality:	284	Length:	225
Ratio:	1.535	Gaps:	3
Percent Similarity:	50.276	Percent Identity:	41.989

Match display thresholds for the alignment(s):

```

| = IDENTITY
: = 2
. = 1

```

FrGcgManager\_77\_MOASSfTL\_ x FrGcgManager\_77\_NOA0mprsU

```

1 CSAQAESCPCTHSPGPRPCPRMDSLQKQDLRRPKIHGAVQASPYQPPT 50
1 .....MSGSFELSVQDLND 14
51 LASLQRLWLVRQAATLNHIDEVWPSLFLGDAYAARDKSKLIQLGITHVVN 100
| | : | | | : | | | | | | | |
15 LLSGSGCYSLPSQPCN...EVTPIRYVGNASVAQDIPKLQKLGITHVLN 61
101 AAAGK..FQVDTGAKFYRGMSLEYGYIEADDNPFDDLVSFVFLPVARYIRA 148
| | : | | | : | | | | | | | |
62 AAEGRSFMHVNTNANFYKDSGITYLGKANDTQEFNLSAYFERAADFIDQ 111
149 ALSVPQGRVLVHCAMGVSRSATLVLAFLMIYENMTLVEAIQTQVQHRNIC 198
| | | | | | | | | | : | | : | |
112 ALAQKNGRVLVHCREGYSRSPTLVIAYLMMRQKMDVKSALSIVRQNRREIG 161
199 PNSGFLRQLQVLDNRLGRDTGRF.. 221
| | | | | | | | : | :
162 PNDGFLAQLCQLNDRLAKE.GKLP 185

```

- 1 -

## SEQUENCE LISTING

&lt;110&gt; Millennium Pharmaceuticals, Inc.

5 &lt;120&gt; NOVEL PROTEIN PHOSPHATASE MOLECULES AND USES THEREFOR

&lt;130&gt; MMI-051CPPC

&lt;140&gt;

10 &lt;141&gt;

&lt;150&gt; USPN 09/164,193

&lt;151&gt; 1999-09-30

15 &lt;150&gt; USPN 09/163,883

&lt;151&gt; 1999-09-30

&lt;160&gt; 11

20 &lt;170&gt; Patent No. 6,111,210

&lt;210&gt; 1

&lt;211&gt; 1315

&lt;212&gt; DNA

25 &lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (249)...(767)

30

&lt;400&gt; 1

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ccccacaccc aagctatttgc acaatatttg tgcgggggtat ggggggtgggt ttttaaattct 120

35

cgtttctctt ggacaagcac agggatctcg ttctcctcat tttttggggg tgtgtgggga 180

cttctcaggt cgtgtcccca gccttctctg cagtcctctc tgccctgccg ggcccgtcgg 240

40

gaggegcc atg gct cgg atg aac cgc ccg gcc ccg gtg gag gtg agc tac 290  
Met Ala Arg Met Asn Arg Pro Ala Pro Val Glu Val Ser Tyr  
1 5 10

45

aaa cac atg cgc ttc ctc atc acc cac aac ccc acc aac gcc acg ctc 338  
Lys His Met Arg Phe Leu Ile Thr His Asn Pro Thr Asn Ala Thr Leu  
15 20 25 30

50

agc acc ttc att gag gac ctg aag aag tac ggg gct acc act gtg gtg 386  
Ser Thr Phe Ile Glu Asp Leu Lys Lys Tyr Gly Ala Thr Thr Val Val  
35 40 45cgt gtg tgt gaa gtg acc tat gac aaa acg ccg ctg gag aag gat ggc 434  
Arg Val Cys Glu Val Thr Tyr Asp Lys Thr Pro Leu Glu Lys Asp Gly  
50 55 60

55

atc acc gtt gtg gac tgg ccg ttt gac gat ggg gcg ccc ccg ccc ggc 482  
Ile Thr Val Val Asp Trp Pro Phe Asp Asp Gly Ala Pro Pro Pro Gly  
65 70 75



aag gta gtg gaa gac tgg ctg agc ctg gtg aag gcc aag ttc tgt gag 530  
 Lys Val Val Glu Asp Trp Leu Ser Leu Val Lys Ala Lys Phe Cys Glu  
 80 85 90

5 gcc ccc ggc agc tgc gtg gct gtg cac tgc gtg gcg ggc ctg ggc cgg 578  
 Ala Pro Gly Ser Cys Val Ala Val His Cys Val Ala Gly Leu Gly Arg  
 95 100 105 110

10 gct cca gtc ctt gtg gcg ctg gcc ctt att gag agc ggg atg aag tac 626  
 Ala Pro Val Leu Val Ala Leu Ala Leu Ile Glu Ser Gly Met Lys Tyr  
 115 120 125

15 gag gac gcc atc cag ttc atc cgc cag aag cgc cgc gga gcc atc aac 674  
 Glu Asp Ala Ile Gln Phe Ile Arg Gln Lys Arg Arg Gly Ala Ile Asn  
 130 135 140

agc aag cag ctc acc tac ctg gag aaa tac cgg ccc aaa cag agg ctg 722  
 Ser Lys Gln Leu Thr Tyr Leu Glu Lys Tyr Arg Pro Lys Gln Arg Leu  
 145 150 155

20 cgg ttc aaa gac cca cac acg cac aag acc cgg tgc tgc gtt atg 767  
 Arg Phe Lys Asp Pro His Thr His Lys Thr Arg Cys Cys Val Met  
 160 165 170

25 tagctcagga ccttggtctgg gcctggctcgt catgtaggtc aggaccttgg ctggacctgg 827  
 aggccctgcc cagccctgct ctgcccagcc cagcaggggc tccaggcctt ggctggcccc 887  
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 ttagaccccg gggcagtcag gtgctccgga caccggaagg caataaaaca ggagccgtga 1247  
 40 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaagg 1307  
 gcggccgn 1315

45 <210> 2  
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 <213> Homo sapiens

50 <400> 2  
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 1 5 10 15

55 Met Arg Phe Leu Ile Thr His Asn Pro Thr Asn Ala Thr Leu Ser Thr  
 20 25 30  
 Phe Ile Glu Asp Leu Lys Lys Tyr Gly Ala Thr Thr Val Val Arg Val  
 35 40 45

Cys Glu Val Thr Tyr Asp Lys Thr Pro Leu Glu Lys Asp Gly Ile Thr  
 50 55 60

5 Val Val Asp Trp Pro Phe Asp Asp Gly Ala Pro Pro Pro Gly Lys Val  
 65 70 75 80

Val Glu Asp Trp Leu Ser Leu Val Lys Ala Lys Phe Cys Glu Ala Pro  
 85 90 95

10 Gly Ser Cys Val Ala Val His Cys Val Ala Gly Leu Gly Arg Ala Pro  
 100 105 110

Val Leu Val Ala Leu Ala Leu Ile Glu Ser Gly Met Lys Tyr Glu Asp  
 115 120 125

15 Ala Ile Gln Phe Ile Arg Gln Lys Arg Arg Gly Ala Ile Asn Ser Lys  
 130 135 140

20 Gln Leu Thr Tyr Leu Glu Lys Tyr Arg Pro Lys Gln Arg Leu Arg Phe  
 145 150 155 160

Lys Asp Pro His Thr His Lys Thr Arg Cys Cys Val Met  
 165 170

25

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 30 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> (1)..(519)

35

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 Met Ala Arg Met Asn Arg Pro Ala Pro Val Glu Val Ser Tyr Lys His  
 1 5 10 15

40 atg cgc ttc ctc atc acc cac aac ccc acc aac gcc acg ctc agc acc 96  
 Met Arg Phe Leu Ile Thr His Asn Pro Thr Asn Ala Thr Leu Ser Thr  
 20 25 30

45 ttc att gag gac ctg aag aag tac ggg gct acc act gtg gtg cgt gtg 144  
 Phe Ile Glu Asp Leu Lys Lys Tyr Gly Ala Thr Thr Val Val Arg Val  
 35 40 45

50 tgt gaa gtg acc tat gac aaa acg ccg ctg gag aag gat ggc atc acc 192  
 Cys Glu Val Thr Tyr Asp Lys Thr Pro Leu Glu Lys Asp Gly Ile Thr  
 50 55 60

gtt gtg gac tgg ccg ttt gac gat ggg gcg ccc ccg ccc ggc aag gta 240  
 Val Val Asp Trp Pro Phe Asp Asp Gly Ala Pro Pro Pro Gly Lys Val  
 55 65 70 75 80

gtg gaa gac tgg ctg agc ctg gtg aag gcc aag ttc tgt gag gcc ccc 288  
 Val Glu Asp Trp Leu Ser Leu Val Lys Ala Lys Phe Cys Glu Ala Pro  
 85 90 95

5 ggc agc tgc gtg gct gtg cac tgc gtg gcg ggc ctg ggc cgg gct cca 336  
 Gly Ser Cys Val Ala Val His Cys Val Ala Gly Leu Gly Arg Ala Pro  
 100 105 110

10 gtc ctt gtg gcg ctg gcc ctt att gag agc ggg atg aag tac gag gac 384  
 Val Leu Val Ala Leu Ala Leu Ile Glu Ser Gly Met Lys Tyr Glu Asp  
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15 gcc atc cag ttc atc cgc cag aag cgc cgc gga gcc atc aac agc aag 432  
 Ala Ile Gln Phe Ile Arg Gln Lys Arg Arg Gly Ala Ile Asn Ser Lys  
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20 cag ctg acc tac ctg gag aaa tac cgg ccc aaa cag agg ctg cgg ttc 480  
 Gln Leu Thr Tyr Leu Glu Lys Tyr Arg Pro Lys Gln Arg Leu Arg Phe  
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40 aac cat gcc gac gag gtc tgg cca ggc ctg tat ctg gga gac cag gac 96  
 Asn His Ala Asp Glu Val Trp Pro Gly Leu Tyr Leu Gly Asp Gln Asp  
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 Gly Leu Gly Ile Arg Tyr Leu Gly Val Glu Ala His Asp Ser Pro Ala  
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60 ttt gac atg agc atc cac ttc cag acg gct gcc gac ttc atc cac cgg 288  
 Phe Asp Met Ser Ile His Phe Gln Thr Ala Ala Asp Phe Ile His Arg  
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65 gcg ctg agc cag cca gga ggg aag atc ctg gtg cat tgt gct gtg ggc 336  
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 Val Ser Arg Ser Ala Thr Leu Val Leu Ala Tyr Leu Met Leu Tyr His  
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5 cac ctt acc ctc gtg gag gcc atc aag aaa gtc aaa gac cac cga gga 432  
 His Leu Thr Leu Val Glu Ala Ile Lys Lys Val Lys Asp His Arg Gly  
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10 gag gcc gag ccc cag gcc act gtc act ctt tgt ggg agg gga cgg gga 480  
 Glu Ala Glu Pro Gln Ala Thr Val Thr Leu Cys Gly Arg Gly Arg Gly  
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15 gtg agg ttg ggc agt gtg gtg gat ggg cac cca gga agg gtt gac cag 528  
 Val Arg Leu Gly Ser Val Val Asp Gly His Pro Gly Arg Val Asp Gln  
 165 170 175

20 gga agg agg cag cta gcc tgt aga tgg aag atg gtc ctg gga ttc gaa 576  
 Gly Arg Arg Gln Leu Gly Cys Arg Trp Lys Met Val Leu Gly Phe Glu  
 180 185 190

25 cac cgc tgg gat ctg gcc agg gtg ctc cct ggg att cac agt ccc ttc 624  
 His Arg Trp Asp Leu Ala Arg Val Leu Pro Gly Ile His Ser Pro Phe  
 195 200 205

30 ccc tct ttg tgc cca agt gtt tcc ctc tct ccc tca cca aaa aca aaa 672  
 Pro Ser Leu Cys Pro Ser Val Ser Leu Ser Pro Ser Pro Lys Thr Lys  
 210 215 220

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 225 230 235 240

40 aag cat gaa tgc aat ggt gta gag ttg tgt gaa acc cct agc ata gag 768  
 Lys His Glu Cys Asn Gly Val Glu Leu Cys Glu Thr Pro Ser Ile Glu  
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45 aca gac agc gaa gag atg gtg tgaaaagctt gcagaaccag acagagaacc 819  
 Thr Asp Ser Glu Glu Met Val  
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50 ccacagactt tccactccaa gcacaggagg aggtagctag cgtgtgaggg ttggcactag 879  
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 5 Met Ala Asn Asn Arg Arg Glu Leu Arg Arg Leu Gly Ile Thr His Val  
                   35                                  40                                  45  
 Leu Asn Ala Ser His Ser Arg Trp Arg Gly Thr Pro Glu Ala Tyr Glu  
                   50                                  55                                  60  
 10 Gly Leu Gly Ile Arg Tyr Leu Gly Val Glu Ala His Asp Ser Pro Ala  
                   65                                  70                                  75                                  80  
 Phe Asp Met Ser Ile His Phe Gln Thr Ala Ala Asp Phe Ile His Arg  
                   85                                  90                                  95  
 15 Ala Leu Ser Gln Pro Gly Gly Lys Ile Leu Val His Cys Ala Val Gly  
                   100                                  105                                  110  
 Val Ser Arg Ser Ala Thr Leu Val Leu Ala Tyr Leu Met Leu Tyr His  
                   115                                  120                                  125  
 His Leu Thr Leu Val Glu Ala Ile Lys Lys Val Lys Asp His Arg Gly  
                   130                                  135                                  140  
 25 Glu Ala Glu Pro Gln Ala Thr Val Thr Leu Cys Gly Arg Gly Arg Gly  
                   145                                  150                                  155                                  160  
 Val Arg Leu Gly Ser Val Val Asp Gly His Pro Gly Arg Val Asp Gln  
                   165                                  170                                  175  
 30 Gly Arg Arg Gln Leu Gly Cys Arg Trp Lys Met Val Leu Gly Phe Glu  
                   180                                  185                                  190  
 His Arg Trp Asp Leu Ala Arg Val Leu Pro Gly Ile His Ser Pro Phe  
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 35 Pro Ser Leu Cys Pro Ser Val Ser Leu Ser Pro Ser Pro Lys Thr Lys  
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 40 Arg Ala Ile Ser Ala Pro Ala Leu Cys Ala Glu Ser Gln Gly Tyr Gly  
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 Asn His Ala Asp Glu Val Trp Pro Gly Leu Tyr Leu Gly Asp Gln Asp  
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10 atg gct aac aac cgc cgg gag ctt cgc cgc ctg ggc atc acg cac gtc 144  
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 35 40 45

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 Leu Asn Ala Ser His Ser Arg Trp Arg Gly Thr Pro Glu Ala Tyr Glu  
 50 55 60

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 Gly Leu Gly Ile Arg Tyr Leu Gly Val Glu Ala His Asp Ser Pro Ala  
 65 70 75 80

25 ttt gac atg agc atc cac ttc cag acg gct gcc gac ttc atc cac cgg 288  
 Phe Asp Met Ser Ile His Phe Gln Thr Ala Ala Asp Phe Ile His Arg  
 85 90 95

30 gcg ctg agc cag cca gga ggg aag atc ctg gtg cat tgt gct gtg ggc 336  
 Ala Leu Ser Gln Pro Gly Gly Lys Ile Leu Val His Cys Ala Val Gly  
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35 gtg agc cga tcc gcc acc ctg gta ctg gcc tac ctc atg ctg tac cac 384  
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40 cac ctt acc ctc gtg gag gcc atc aag aaa gtc aaa gac cac cga gga 432  
 His Leu Thr Leu Val Glu Ala Ile Lys Lys Val Lys Asp His Arg Gly  
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45 gag gcc gag ccc cag gcc act gtc act ctt tgt ggg agg gga cgg gga 480  
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50 gtg agg ttg ggc agt gtg gtg gat ggg cac cca gga agg gtt gac cag 528  
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55 gga agg agg cag cta ggc tgt aga tgg aag atg gtc ctg gga ttc gaa 576  
 Gly Arg Arg Gln Leu Gly Cys Arg Trp Lys Met Val Leu Gly Phe Glu  
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60 cac cgc tgg gat ctg gcc agg gtg ctc cct ggg att cac agt ccc ttc 624  
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65 ccc tct ttg tgc cca agt gtt tcc ctc tct ccc tca cca aaa aca aaa 672  
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25

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35

40

45

50

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tgg cgc ggg tgc tct tct acc cga cgc tgc tct aca ccc tgt tcc gcg  
Trp Arg Gly Cys Ser Ser Thr Arg Arg Cys Ser Thr Pro Cys Ser Ala 96

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5 cca ccg tgc tgc tgg gcg cgc tgc cgt tgc gga agc ttg acg cgc cag 192  
 Pro Pro Cys Cys Trp Ala Arg Cys Arg Cys Gly Ser Leu Thr Arg Gln  
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10 ctg gta cag gac gag aac gtg cgc ggg gtg atc acc atg aac gag gag 240  
 Leu Val Gln Asp Glu Asn Val Arg Gly Val Ile Thr Met Asn Glu Glu  
 65 70 75 80

15 tac gag acg agg ttc ctg tgc aac tct tca cag gag tgg aag aga cta 288  
 Tyr Glu Thr Arg Phe Leu Cys Asn Ser Ser Gln Glu Trp Lys Arg Leu  
 85 90 95

20 gga gtc gag cag ctg cgg ctc agc aca gta gac atg act ggg atc ccc 336  
 Gly Val Glu Gln Leu Arg Leu Ser Thr Val Asp Met Thr Gly Ile Pro  
 100 105 110

acc ttg gac aac ctc cag aag gga gtc caa ttt gct ctc aag tac cag 384  
 Thr Leu Asp Asn Leu Gln Lys Gly Val Gln Phe Ala Leu Lys Tyr Gln  
 115 120 125

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 Ser Leu Gly Gln Cys Val Tyr Val His Cys Lys Ala Gly Arg Ser Arg  
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30 agt gcc act atg gtg gca gca tac ctg att cag gtt gca caa atg gga 480  
 Ser Ala Thr Met Val Ala Ala Tyr Leu Ile Gln Val Ala Gln Met Gly  
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35 gtc cag agg agg ctg nnn gag cca tcg ccc aag atc cgg gtc ata cat 528  
 Val Gln Arg Arg Leu Xaa Glu Pro Ser Pro Lys Ile Arg Val Ile His  
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cca cat cag gcc tgg cca gct tgg atg ttc tta aag agt tcc aca aag 576  
 Pro His Gln Ala Trp Pro Ala Trp Met Phe Leu Lys Ser Ser Thr Lys  
 180 185 190

40 cag att act gca csg gcm aca aag gat ggg act ttt gkc att tca aag 624  
 Gln Ile Thr Ala Xaa Xaa Thr Lys Asp Gly Thr Phe Xaa Ile Ser Lys  
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45 aca tgatgtatgg ggattagaaa gaactcaaga cactcctgct tgatacagaa 677  
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10 Pro Pro Cys Cys Trp Ala Arg Cys Arg Cys Gly Ser Leu Thr Arg Gln  
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15 Leu Val Gln Asp Glu Asn Val Arg Gly Val Ile Thr Met Asn Glu Glu  
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Tyr Glu Thr Arg Phe Leu Cys Asn Ser Ser Gln Glu Trp Lys Arg Leu  
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20 Gly Val Glu Gln Leu Arg Leu Ser Thr Val Asp Met Thr Gly Ile Pro  
 100 105 110

Thr Leu Asp Asn Leu Gln Lys Gly Val Gln Phe Ala Leu Lys Tyr Gln  
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25 Ser Leu Gly Gln Cys Val Tyr Val His Cys Lys Ala Gly Arg Ser Arg  
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30 Ser Ala Thr Met Val Ala Ala Tyr Leu Ile Gln Val Ala Gln Met Gly  
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Val Gln Arg Arg Leu Xaa Glu Pro Ser Pro Lys Ile Arg Val Ile His  
 165 170 175

35 Pro His Gln Ala Trp Pro Ala Trp Met Phe Leu Lys Ser Ser Thr Lys  
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Gln Ile Thr Ala Xaa Xaa Thr Lys Asp Gly Thr Phe Xaa Ile Ser Lys  
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 Trp Arg Gly Cys Ser Ser Thr Arg Arg Cys Ser Thr Pro Cys Ser Ala  
 20 25 30

35 gga agg tgc cgg gtc ggg cgc acc ggg act ggt acc acc gca tgc acc 144  
 Gly Arg Cys Arg Val Gly Arg Thr Gly Thr Gly Thr Thr Ala Ser Thr  
 35 40 45

40 cca ccg tgc tgc tgg gcg cgc tgc cgt tgc gga agc ttg acg cgc cag 192  
 Pro Pro Cys Cys Trp Ala Arg Cys Arg Cys Gly Ser Leu Thr Arg Gln  
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45 ctg gta cag gac gag aac gtg cgc ggg gtg atc acc atg aac gag gag 240  
 Leu Val Gln Asp Glu Asn Val Arg Gly Val Ile Thr Met Asn Glu Glu  
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 Tyr Glu Thr Arg Phe Leu Cys Asn Ser Ser Gln Glu Trp Lys Arg Leu  
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 Gly Val Glu Gln Leu Arg Leu Ser Thr Val Asp Met Thr Gly Ile Pro  
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tcg ctg ggc cag tgt gtt tac gtg cat tgt aag gct ggg cgc tcc agg 432  
 Ser Leu Gly Gln Cys Val Tyr Val His Cys Lys Ala Gly Arg Ser Arg  
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 Ser Ala Thr Met Val Ala Ala Tyr Leu Ile Gln Val Ala Gln Met Gly  
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5 gtc cag agg agg ctg nnn gag cca tcg ccc aag atc cgg gtc ata cat 528  
 Val Gln Arg Arg Leu Xaa Glu Pro Ser Pro Lys Ile Arg Val Ile His  
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10 cca cat cag gcc tgg cca gct tgg atg ttc tta aag agt tcc aca aag 576  
 Pro His Gln Ala Trp Pro Ala Trp Met Phe Leu Lys Ser Ser Thr Lys  
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15 cag att act gca csg gcm aca aag gat ggg act ttt gkc att tca aag 624  
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 Gly Pro Arg Pro Cys Pro Arg Met Asp Ser Leu Gln Lys Gln Asp Leu  
 20 25 30

40 cgg agg ccc aag atc cat ggg gca gtc cag gca tct ccc tac cag ccg 144  
 Arg Arg Pro Lys Ile His Gly Ala Val Gln Ala Ser Pro Tyr Gln Pro  
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45 ccc aca ttg gct tcg ctg cag cgc ttg ctg tgg gtc cgt cag gct gcc 192  
 Pro Thr Leu Ala Ser Leu Gln Arg Leu Leu Trp Val Arg Gln Ala Ala  
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50 aca ctg aac cat atc gat gag gtc tgg ccc agc ctc ttc ctg gga gat 240  
 Thr Leu Asn His Ile Asp Glu Val Trp Pro Ser Leu Phe Leu Gly Asp  
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55 gcg tac gca gcc cgg gac aag agc aag ctg atc cag ctg gga atc acc 288  
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cac gtt gtg aat gcc gct gca ggc aag ttc cag gtg gac aca ggt gcc 336  
 His Val Val Asn Ala Ala Ala Gly Lys Phe Gln Val Asp Thr Gly Ala  
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 Lys Phe Tyr Arg Gly Met Ser Leu Glu Tyr Tyr Gly Ile Glu Ala Asp  
 115 120 125

5 gac aac ccc ttc ttc gac ctc agt gtc tac ttt ctg cct gtt gct cga 432  
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10 tac atc cga gct gcc ctc agt gtt ccc caa ggc cgc gtg ctg gta cac 480  
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 145 150 155 160

15 tgt gcc atg ggg gta agc cgc tct gcc aca ctt gtc ctg gcc ttc ctc 528  
 Cys Ala Met Gly Val Ser Arg Ser Ala Thr Leu Val Leu Ala Phe Leu  
 165 170 175

atg atc tat gag aac atg acg ctg gta gag gcc atc cag acg gtg cag 576  
 Met Ile Tyr Glu Asn Met Thr Leu Val Glu Ala Ile Gln Thr Val Gln  
 180 185 190

20 gcc cac cga aat atc tgc cct aac tca ggc ttc ctc cgg cag ctc cag 624  
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 195 200 205

25 gtt ctg gac aac cga ctg ggg cgg gac acg ggg cgg ttc tgatctggca 673  
 Val Leu Asp Asn Arg Leu Gly Arg Asp Thr Gly Arg Phe  
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30 ggcagccagg atccctgacc cttggcccaa cccaccagc ctggccctgg gaacagcagg 733  
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55 Pro Thr Leu Ala Ser Leu Gln Arg Leu Leu Trp Val Arg Gln Ala Ala  
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Thr Leu Asn His Ile Asp Glu Val Trp Pro Ser Leu Phe Leu Gly Asp  
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Ala Tyr Ala Ala Arg Asp Lys Ser Lys Leu Ile Gln Leu Gly Ile Thr  
85 90 95

5 His Val Val Asn Ala Ala Ala Gly Lys Phe Gln Val Asp Thr Gly Ala  
100 105 110

Lys Phe Tyr Arg Gly Met Ser Leu Glu Tyr Tyr Gly Ile Glu Ala Asp  
115 120 125

10 Asp Asn Pro Phe Phe Asp Leu Ser Val Tyr Phe Leu Pro Val Ala Arg  
130 135 140

Tyr Ile Arg Ala Ala Leu Ser Val Pro Gln Gly Arg Val Leu Val His  
15 145 150 155 160

Cys Ala Met Gly Val Ser Arg Ser Ala Thr Leu Val Leu Ala Phe Leu  
165 170 175

20 Met Ile Tyr Glu Asn Met Thr Leu Val Glu Ala Ile Gln Thr Val Gln  
180 185 190

Ala His Arg Asn Ile Cys Pro Asn Ser Gly Phe Leu Arg Gln Leu Gln  
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45 ggc ccc agg cca tgc ccc agg atg gac tca ctg cag aag cag gac ctc 96  
Gly Pro Arg Pro Cys Pro Arg Met Asp Ser Leu Gln Lys Gln Asp Leu  
20 25 30

50 cgg agg ccc aag atc cat ggg gca gtc cag gca tct ccc tac cag ccg 144  
Arg Arg Pro Lys Ile His Gly Ala Val Gln Ala Ser Pro Tyr Gln Pro  
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ccc aca ttg gct tcg ctg cag cgc ttg ctg tgg gtc cgt cag gct gcc 192  
Pro Thr Leu Ala Ser Leu Gln Arg Leu Leu Trp Val Arg Gln Ala Ala  
50 55 60

55 aca ctg aac cat atc gat gag gtc tgg ccc agc ctc ttc ctg gga gat 240  
Thr Leu Asn His Ile Asp Glu Val Trp Pro Ser Leu Phe Leu Gly Asp  
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	85 90 95	
5	cac gtt gtg aat gcc gct gca ggc aag ttc cag gtg gac aca ggt gcc	336
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	100 105 110	
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	115 120 125	
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	Asp Asn Pro Phe Phe Asp Leu Ser Val Tyr Phe Leu Pro Val Ala Arg	
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	Tyr Ile Arg Ala Ala Leu Ser Val Pro Gln Gly Arg Val Leu Val His	
	145 150 155 160	
25	tgt gcc atg ggg gta agc cgc tct gcc aca ctt gtc ctg gcc ttc ctc	528
	Cys Ala Met Gly Val Ser Arg Ser Ala Thr Leu Val Leu Ala Phe Leu	
	165 170 175	
30	atg atc tat gag aac atg acg ctg gta gag gcc atc cag acg gtg cag	576
	Met Ile Tyr Glu Asn Met Thr Leu Val Glu Ala Ile Gln Thr Val Gln	
	180 185 190	
35	gcc cac cgc aat atc tgc cct aac tca ggc ttc ctc cgg cag ctc cag	624
	Ala His Arg Asn Ile Cys Pro Asn Ser Gly Phe Leu Arg Gln Leu Gln	
	195 200 205	
40	gtt ctg gac aac cga ctg ggg cgg gac acg ggg cgg ttc	663
	Val Leu Asp Asn Arg Leu Gly Arg Asp Thr Gly Arg Phe	
	210 215 220	

# INTERNATIONAL SEARCH REPORT

Internat. Application No

PCT/US 99/22924

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/55 C12N9/16 C12N5/10 C07K16/40 G01N33/573  
C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 14596 A (INCYTE PHARMA INC ;GOLI SURYA K (US)) 9 April 1998 (1998-04-09) abstract page 2, line 31 -page 3, line 3 page 20, line 12 -page 21, line 24 page 22, line 22 - line 32 seq id nos 3,4	1-3,5-22
Y	---	1-22
Y	WO 94 03611 A (MAX PLANCK GESELLSCHAFT) 17 February 1994 (1994-02-17) abstract page 40, line 29 -page 41, line 2 page 50, line 15 -page 51, line 13 ---	1-22
	--- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*Z\* document member of the same patent family

Date of the actual completion of the international search

8 March 2000

Date of mailing of the international search report

07.06.2000

Name and mailing address of the ISA

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Authorized officer

CEDER O.

## INTERNATIONAL SEARCH REPORT

Internat. Application No.

PCT/US 99/22924

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZENG ET AL.: "Homo sapiens potentially prenylated protein tyrosine phosphatase" EMBL SEQUENCE DATABASE, 10 August 1998 (1998-08-10), XP002132488 HEIDELBERG DE AC AF041434 the whole document	1,2,8,9
P,X	-& ZENG ET AL.: "HPRL-3" EMBL SEQUENCE DATABASE, 1 November 1998 (1998-11-01), XP002132489 HEIDELBERG DE Ac 075365 the whole document	8,9
X	--- ZENG ET AL.: "Protein tyrosine phosphatase 4A3 (MPRL-3)" EMBL SEQUENCE DATABASE, 1 August 1998 (1998-08-01), XP002132490 HEIDELBERG DE Ac 070275 the whole document	8,9
A	-& ZENG ET AL.: "Mus musculus potentially prenylated protein tyrosine phosphatase mPRL-3 (Pr13) mRNA, complet compound" EMBL SEQUENCE DATABASE, 30 March 1998 (1998-03-30), XP002132491 HEIDELBERG DE Ac AF035645 the whole document & ZENG ET AL.: "Mouse PRL-2 and PRL-3, two potentially prenylated protein tyrosine phosphatases homologous to PRL-1" BIOCHEM BIOPHYS RES COMMUN, vol. 244, 1998, pages 421-427, -----	1,2,8,9



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 99/22924

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
Claims 1-22, as far as they relate to "the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_", could not be searched since the accession numbers were not disclosed, neither in the claims nor in the description.
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-22 all partly

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims 1-22, as far as they relate to "the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, or \_\_\_\_\_", could not be searched since the accession numbers were not disclosed, neither in the claims nor in the description.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-22 all partly

An isolated nucleic acid molecule, an isolated polypeptide, an antibody, a vector and a host cell and their uses all related to the CSAPTP sequences with SEQ ID NOS 1-3.

2. Claims: 1-22 all partly

An isolated nucleic acid molecule, an isolated polypeptide, an antibody, a vector and a host cell and their uses all related to the CSAPTP sequences with SEQ ID NOS 4-6.

3. Claims: 1-22 all partly

An isolated nucleic acid molecule, an isolated polypeptide, an antibody, a vector and a host cell and their uses all related to the CSAPTP sequences with SEQ ID NOS 7-9.

4. Claims: 1-22 all partly

An isolated nucleic acid molecule, an isolated polypeptide, an antibody, a vector and a host cell and their uses all related to the CSAPTP sequences with SEQ ID NOS 10-12.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No

PCT/US 99/22924

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9814596	A	09-04-1998	US	6020179 A	01-02-2000
			AU	4667397 A	24-04-1998
			EP	0951556 A	27-10-1999
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WO 9403611	A	17-02-1994	AU	4707793 A	03-03-1994
			CA	2141847 A	17-02-1994
			CN	1091139 A	24-08-1994
			EP	0654083 A	24-05-1995
			JP	8501934 T	05-03-1996
			MX	9304769 A	31-05-1994
			US	5955592 A	21-09-1999
			US	5831009 A	03-11-1998
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